

UNIVERSITY OF BRISTOL



THE ANNUAL REPORT

OF THE

Agricultural and Horticultural
Research Station

(THE NATIONAL FRUIT AND CIDER INSTITUTE)

LONG ASHTON, BRISTOL
1950

BATH :

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* Agricultural Research Council Plant Nutrition Scheme.

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INTRODUCTION.

By T. WALLACE, C.B.E., M.C., D.Sc., F.R.I.C., Director.

During 1950 the post-war programme of investigations has proceeded without serious interruption and good progress can be recorded in the various branches of the work. It has, however, been necessary to give special attention to economies in administration to help in the Government's efforts to reduce inflationary pressure and, in this connection, every effort has been made to comply with the policy of the Ministry of Agriculture to effect economies without impairing the progress of research.

Liaison with the local officers of the National Agricultural Advisory Service in their advisory work has been effectively maintained by personal visits of the officers to the Station in connection with enquiries and by their attendance, by invitation, at staff meetings. In addition, relationships have been further strengthened with the Service by the decision of the Ministry of Agriculture to hold annual refresher courses for N.A.A.S. horticultural officers at Long Ashton, the second of which was held in July, and by the representation of the Station on the governing bodies of the recently established N.A.A.S. Horticulture Experimental Stations.

Good progress has been made during 1950 in developing experimental work at one of these stations at Luddington, near Stratford-on-Avon, Warwickshire, where the Station is collaborating in a number of joint field experiments. These stations should prove of great value to the Institute in its field work, as the problem of finding further new land on the Station farm or near Long Ashton, has become impossible of solution. The Institute is collaborating with the East Malling Research Station in providing material for planting at the new Stations.

On November 15th and 16th a course in pruning for N.A.A.S. officers was given by Mr. F. A. Roach, Provincial Fruit Specialist for the South-Western Province of the N.A.A.S. and Mr. Hobbs, Station Plantations Officer.

A further step to establish still closer relationships with N.A.A.S. officers and with growers has been taken by appointing to the Institute staff a Public Relations Officer, who will function as the head of a technical information section to make known to growers and the general public results of research work, by the distribution of publications and the organising of demonstrations and open days at the Institute and lectures to growers' organisations by members of the staff. It is hoped that, as the work in the new section develops, it will be possible to keep growers and the general public well informed of the progress of research and ensure that results of practical importance are applied without undue delay.

It will be recalled that this appointment had been postponed owing to lack of accommodation. It has only been possible to make the appointment at the present stage by the absence of the Willow Officer on National Service, the Public Relations Officer having taken over the vacant accommodation as a temporary measure.

The new University Horticultural Diploma course for graduates began in October. The students attend at Long Ashton for lectures and practical work in subjects connected with Fruit Culture, Pests and Diseases, and Soils and Manures.

On October 17th, Dr. W. K. Slater, Secretary of the Agricultural

Research Council, visited the Institute to discuss with the Director and members of staff various problems concerning the research programme and accommodation. Following this visit a long-term development project for buildings has been formulated and submitted to the Agricultural Research Council for consideration.

The Cider Advisory Committee and its two sub-committees all met during the year. The main Committee and the Orchardring Sub-Committee held a joint meeting on May 4th, the main business being to consider the programmes of work in Cider Making and Cider Orchardring for the year 1950/51 and the progress of the scheme for Cider Variety Trial Plots. In the latter connection it was reported that 24 offers of sites had been received, representing the counties of Hereford, Somerset, Gloucester, Devon, Wiltshire, Monmouth, Dorset and Essex. Subsequent to the meeting the sites were inspected for suitability, also further sites proposed in Worcestershire, and, so far, sites have been approved as follows and agreements reached with the owners of the respective sites in accordance with the conditions of the scheme.

Standard orchards : Devon 2, Hereford 1, Somerset 2.

Bush plantations : Devon 1, Essex 1, Gloucester 1, Hereford 3, Somerset 1, Worcester 1.

At the above meeting it was also decided to revise the membership of the Orchardring Sub-Committee in view of the changes in county staffs following the establishment of the N.A.A.S. The new membership provides for adequate representation of interested counties and the N.A.A.S.

At the meeting of the Cider Manufacture Sub-Committee on July 20th, detailed proposals for the research programme on cider making for 1950/51 were discussed. It was also decided that future annual meetings of the Sub-Committee should be held in the month of July.

During the year arrangements were made by the University to include on the Agricultural Committee representatives of the Food Investigation Board, the National Cider Makers' Association and the Apple Juice Producers' Association of Great Britain, all three bodies being closely associated with the work of the Cider and Fruit Juices section and having representatives on the Agricultural Research Council committee responsible for the supervision of the work of the Section.

The Agricultural Committee during the year has lost the services of three valued members. Mr. Richard Stratton, who had represented Wiltshire County Council for many years, resigned his membership on 20/1/50 because of ill-health and it was with great regret that we learned of his death later in the year. The vacancy caused by Mr. Stratton's resignation has been filled by Mr. O. M. Peall, whom we are pleased to welcome to the Committee.

Mr. Ronald Whiteway, who had been a Governor of the National Fruit and Cider Institute since 1945 and had represented the Institute on the Agricultural Committee from 1947, died in early January, 1951. Mr. Whiteway had been in poor health for a considerable period but had nevertheless kept in touch with the work of the Institute. His loss will be much felt by the Committee in view of his great knowledge of the cider industry.

Mr. R. H. Densham, who has been associated with the cider work of the Institute for many years and who served on the Governing Body as a representative of Devon County Council, and on the Agricultural Committee as a representative of the Institute, resigned on 23rd October, 1950, on giving up his membership of Devon County committees on medical advice. Mr. Densham

has always been a keen supporter of the cider fruit growers and his views on their problems have always been valued by the Institute. We trust that Mr. Densham's health will improve and that we shall continue to see him at the Institute on Open Days.

The Committee has also been without the services of Captain Andrew Haggard for some months due to a severe illness. Captain Haggard is one of our oldest Committee members and we all look forward to seeing him once more among us, fully recovered, and taking part in our work with all his old vigour and enthusiasm.

It is with regret that we also record the death of Mr. Herbert Watts on February 16th, 1950. Many members will remember Mr. Watts as the Institute Cider Maker from 1904 until 1924. He left Long Ashton to become manager of the Gloucestershire Cider Co., Wickwar, and subsequently became Managing Director.

Difficulty was again experienced in obtaining candidates for the Memorial Scholarship in cider making and cider orcharding to commemorate the services to the Institute of Messrs. John Wootton, Eldred Walker and John Ettle. The award was eventually made to Mr. W. H. Evans, a nominee of the Monmouthshire County Council. Mr. Evans began his training on 1st October, 1950, and is making good progress. It is hoped that more applications will be received for the 1951 award.

Staff.

There have been some important staff changes during the year, following on the resignations of Dr. H. Martin and Dr. S. W. Challinor, both of whom were heads of sections. Dr. Challinor's resignation, in particular, has necessitated a regrouping of the staff of the Cider and Fruit Juices Sections. With the agreement of the Agricultural Research Council, these two sections, whose researches are closely related, were joined together under one head, and Dr. A. Pollard, previously head of the Fruit Juices Section, was appointed as head of the new section. Dr. Pollard is well fitted by training and experience to undertake his new responsibility, as he was associated with both cider and fruit juices research under Dr. Charley, when the two sections were previously run as a combined unit. The resignation of Mr. R. C. Codner from the post of microbiologist in the Cider Section called for further reorganisation to cover the various subjects, but this has been satisfactorily accomplished by the transfer of Mr. F. W. Beech from biochemistry to microbiology. Mr. Beech will also take over charge of experiments and the supervision of technical operations in the Cider House.

The part-time appointment of Professor Barker in the Cider Section was terminated on 31/12/50. Professor Barker had held this appointment, since resigning from the post of Director in 1943, to help in building up a new research unit and with advisory work. The thanks of the Institute are due to him for the advice and guidance he has given during this period of reconstruction. He will not be leaving the Station, as arrangements have been made for him to continue his research work in an honorary capacity as a Professor Emeritus.

Dr. Hubert Martin relinquished his appointment on August 31st to take up the post of Director of the Science Service Pathology Laboratory of the Canadian Department of Agriculture, University of Western Ontario, London, Ontario, Canada. Dr. Martin was appointed to the Station staff, as head of the section of Insecticides and Fungicides, in December, 1933. His long

service at the Station was outstanding from several viewpoints. His work on insecticides and fungicides at Long Ashton had gained for him an international reputation in these fields and he contributed much to the knowledge of the practical methods of pest and disease control in fruit and other crops. His scientific contributions received recognition by the University in 1948 by his appointment to a Readership in Biochemistry.

Research workers, students and practical growers are all indebted to Dr. Martin for his invaluable book "The Scientific Principles of Plant Protection," which is a standard text book in the subject. Dr. Martin's services were in great demand on Government Committees and he played an important part in establishing national standards for insecticides. He will always be remembered by his colleagues, especially the younger ones, for his helpful guidance and criticism in their work and for his good fellowship. On leaving to take up his new post, he carries with him the very best wishes for his future success from the University Agricultural Committee, the governors and members of the Institute and his colleagues on the staff.

Appointments. Dr. A. Pollard was appointed as Head of the new Cider and Fruit Juices section from 1/1/51.

Mr. C. F. Timberlake was appointed as Organic Chemist in the Fruit Juices Section from 1/3/50. He is a graduate of the University of Wales and since leaving the University has held three chemical posts, the last as Assistant Analyst to Glamorgan County Public Analyst.

Dr. R. J. W. Byrde was appointed to the post of Assistant Mycologist on 1/11/50. Prior to his appointment Dr. Byrde had been working for his Doctorate of Reading University at Long Ashton since 1947 under the guidance of Mr. Marsh. During that time he had carried out investigations on Brown Rot of apples and pears. From 1943 to 1947 Dr. Byrde had served in the R.E.M.E. as a Radar Maintenance Officer in India, leaving with the rank of Captain.

Mr. H. S. D. Swan was appointed as Public Relations Officer from 11/12/50. Mr. Swan is a graduate in Economics of Cambridge University and in Forestry of Oxford University. He served in the army from 1939 to 1946, and saw service as an artillery officer in the S.E.A.C. in India, Ceylon, Burma, Malaya and Java. He later held the appointment of Staff Captain in an Indian Air Liaison Section.

Mr. A. I. Campbell was appointed to take charge of the Station nurseries from 1/11/50. Mr. Campbell was trained in the R.H.S. Gardens and has had practical experience in commercial nurseries. Prior to joining the Long Ashton staff he was employed as Fruit Officer to the Royal Horticultural Society. He served from 1941 to 1945 in the R.A.F. with the rank of Sergeant-Instructor.

Appointments to, and promotions of, assistant staff were as follows :—

Miss J. L. Allan, Assistant (Scientific), Pomology, from 24/4/50.

Mr. R. A. Coggins, Assistant (Scientific), Cider, from 1/5/50.

Mr. H. J. Jones, Station Workshops, from 1/2/50.

Miss P. M. James, Assistant (Scientific), Organic Chemistry, to Assistant Experimental Officer from 1/10/50.

Miss M. R. Moseley, Assistant (Scientific), Fruit Juices, to Assistant Experimental Officer from 1/10/50.

Resignations. There were three resignations from the scientific staff during the year.

Dr. H. Martin resigned from 31/8/50 to take up the post of Director,

Science Service Laboratory, Canadian Department of Agriculture, University of Western Ontario, Canada.

Dr. S. W. Challinor resigned from 31/12/50 to take up an appointment as Senior Lecturer in Microbiology, Department of Industrial Fermentation, University of Birmingham.

Professor B. T. P. Barker relinquished his part-time appointment in the Cider section from 31/12/50.

The following members of the assistant staff resigned their posts during the year.

Mr. J. W. Bultitude resigned from 31/8/50 following a period of ill-health.

Miss C. E. C. Clarke, Recorder for Spraying Experiments, from 6/1/50.

Mr. J. B. White, Assistant (Scientific), Cider, from 31/10/50.

Temporary Workers. The following have been accommodated in the Institute laboratories during the year.

Dr. H. Gleen, of the Agricultural Research Council's team of workers on soil metabolism, continued his work throughout the year.

Miss H. M. Ferres, M.Sc., Adelaide, who had held the appointment of Plant Physiologist at the Waite Institute, Adelaide, has worked in the Pomology section from 13/2/50, under a special grant from the Agricultural Research Council to investigate problems of "The accumulation of growth inhibiting substances in buds in relation to the winter dormancy of fruit trees."

Miss N. F. Millis has continued her investigations, as Boots Research Scholar, in the Cider section.

Miss P. Miles, of the N.A.A.S., has continued her investigations of bulb nutrition in sand cultures under Dr. E. J. Hewitt.

Mr. E. J. Winter, of the National Vegetable Research Station, concluded his studies in sand culture methods and left the Station on 12/8/50.

Visits Abroad. Reference was made in the 1949 Annual Report to the visit of Mr. R. W. Marsh to Canada and the U.S.A. for a period of one year in exchange with Dr. R. S. Willison, Plant Pathology Laboratory, Ontario, and aided by a grant from the Agricultural Research Council. A summary was also included of Mr. Marsh's main activities during his stay and mention was made of a report submitted to the Ministry of Agriculture. The report has now been duplicated by the Agricultural Research Council and widely circulated to research workers and others interested in the modern development of insecticides and fungicides in North America. Mr. Marsh returned to Long Ashton on March 9th, 1950.

A number of other visits to countries in Europe were made during 1950 by members of staff.

Mr. F. W. Beech took part in the tour organised by the National Association of Cider Makers to cider producing areas in northern France from May 16th-22nd. During the tour Mr. Beech visited a number of cider factories and was able to study the latest methods used in cider making. He also visited a number of cider apple nurseries and factories producing cider-making equipment.

Dr. A. Pollard attended the 2nd International Congress of Fruit Juice Producers at Zurich from May 30th-June 6th. Attendance at this Congress enabled Dr. Pollard to judge the state of the industry in the various European countries. There is a lively interest in fruit beverages in several countries, but it is recognised that there are serious difficulties to be overcome to expand their consumption.

Dr. D. J. D. Nicholas attended the 7th International Botanical Congress

in Stockholm, at which he contributed two papers, from July 12th–20th. He afterwards visited centres in Finland, Denmark, Holland and France. The Congress in Stockholm was a very large one, being attended by over 1500 delegates from 36 countries. The contacts made and experiences gained by Dr. Nicholas, both during the Congress and on his subsequent tour, should prove very useful in the development of his investigations in plant nutrition. Centres visited included the Royal Agricultural College, Ultuna, and University of Uppsala, Sweden; University of Helsinki and the Agricultural Experiment Station, Tikkurila, Finland; Phytopathology Station, Lyngby, Denmark; and the Pasteur Institute and the Sorbonne, Paris.

The Director and Dr. C. Bould attended the 4th International Congress of Soil Science in Amsterdam from July 24th–August 1st and took part in a post-Congress tour to horticultural centres around the town of Breda. The Director delivered one of the two Congress lectures given at the plenary sessions of the Congress and a paper was also read at a sectional meeting by Dr. Bould. During the post-Congress tour the important horticultural centre of Westland was visited. An unofficial visit was also paid to the fruit growing districts around Wageningen.

Mr. S. H. Crowdy received an invitation from the Rhode Island Agricultural College, U.S.A., to spend a period of 9 months, beginning October 1st, 1950, at the College to collaborate in research work in plant chemotherapy. With the concurrence of the A.R.C. and the University, leave of absence was given to Mr. Crowdy to enable him to accept this generous offer. The work which Mr. Crowdy will carry out at Rhode Island is a natural extension of the important work he had recently initiated at Long Ashton on systemic fungicides.

Buildings and Land.

The small special building for water distillation and the preparation of pots and sand for pot culture experiments was completed in March in time for the season's work. A portion of the old pot culture shed was also adapted for work on the preparation of nutrient solutions and has been fully utilised during the year. Two small aluminium greenhouses, for work on radio-active tracer elements and growth-regulating substances, were completed during the summer and have been in full use.

The laying of the dust-free floor in the large pot culture greenhouse was completed in spring and has greatly improved conditions for experiments on trace elements. It has not yet been possible to treat the surface of the pot culture enclosure, but this is due to receive attention at an early date. The erection of a wire fence around this area has been postponed pending the completion of other work in the area.

A comprehensive development scheme for buildings and greenhouses has been drawn up. It comprises a main laboratory building, including office accommodation and a canteen, a greenhouse block with rooms attached for special experimental work requiring controlled environmental conditions, farm buildings, a fruit store, stores for the Biology Laboratory, some minor alterations to the present laboratories and a cottage to be adapted from the present offices. The pilot factory building for cider and fruit juices will also be considered as part of this main scheme. Since under present conditions it is unlikely that the scheme can be proceeded with in full for a considerable time, priorities have been decided upon to meet the most urgent needs.

There has again been little change on the farm during the year. A special nursery has been established to provide scion wood of all the cider varieties

in the recommended lists. In the tree nursery the stocks for the cider trial trees, both for bushes and standards, were budded during the year. The stocks used are seedling crab and vegetatively raised Crab "C." Varieties of black currants and gooseberries are also being propagated to provide planting material for the experimental plots of the N.A.A.S. Experimental Horticulture farms.

Black currant crops were again very heavy, but tree crops—apples and pears—although initially heavy were severely damaged by violent gales and the harvested crops were light. The wet summer and autumn also made harvesting difficult and on the whole the year's results were very disappointing. For the second successive season there was a heavy crop of cider apples throughout the south-west area and this, together with the heavy crops of windfalls from commercial orchards, made the sale of cider fruit particularly difficult.

Research Activities.

Plant Nutrition. The main studies in fruit tree nutrition were in continuation of 1949 experiments. In cultural experiments on apples, trees under permanent grass cover again showed low nitrogen, high phosphorus and high potassium status. Yields of one variety were significantly higher under grass than arable or cover crop systems. Urea foliage sprays did not affect the nitrogen content of apple leaves in a season of high rainfall, the N content for unsprayed trees being 2% of dry matter. In a comparison of methods of applying magnesium to deficient apple trees, foliage sprays were effective in the season of application, whereas soil applications to the surface and subsoil (by irrigation) were without effect.

On black currants, leaf nitrogen status decreased according to the following order of manurial treatments: NP > sewage sludge > nitro chalk plus compost > straw sludge compost > FYM > no manure. Yields followed the order of nitrogen content of the foliage. Copper and zinc deficiencies of apples and pears were diagnosed at several new centres. Pears have shown a high susceptibility to copper deficiency and effects are very serious. Foliage sprays of 0.1% ZnSO_4 plus spreader applied at pink bud stage and of 0.05% CuSO_4 plus lime and spreader have given effective control of the two deficiencies. Foliage sprays of borax applied at a late stage, branch injection of boric acid and soil dressings of borax all raised the boron content of apple fruits but had no effect on bitter pit.

In chromatographic studies of the phenolic constituents of pear trees marked varietal differences were noted. In the bark of Conference *l*-epicatechin is dominant and in Williams *d*-catechin. The glucoside arbutin, typical of pears, is absent from the leaves and bark of quince. The main phenolic constituent of apple and pear juice appears to be chlorogenic acid. It is also prominent in the pear leaves and occurs in the bark. Its presence in apple leaves and bark tissues seems doubtful.

In sand culture investigations factorial experiments were carried out on P/Mn/Al relationships in the soil acidity complex and Fe/K/P relationships in the nutrition of the potato. In these experiments 1949 results were generally confirmed both as regards qualitative and quantitative effects. Experiments on the effect of heavy metals on the iron status of plants were made in sand and soil cultures. In the former the effects of nitrogen source (nitrate and urea) and molybdenum level were examined. Molybdenum generally accentuated the toxic effects of the heavy metals, particularly in the urea

series. In the nitrate series high Mo was associated with reduced effects in the case of sodium chromate.

In the soil cultures (pH 5.3) Cu, Zn, Co and Ni at 10 milliequivalents all induced a deficiency of iron in tomato, but Mn and Pb produced no visible injury. With oats on this soil, Cu at 70 milliequivalents reduced the yield of tops.

The chemical analysis of plant material from previous tests showed no consistent relationship between iron content and heavy metal effects. Generally the heavy metals raised the iron content in chlorotic plants. Chlorophyll contents paralleled the chlorotic effects, the relationship being an inverse one.

In further studies of molybdenum nutrition, the range of visual symptoms and yield effects was determined for a number of Brassica crops. The order of decreasing susceptibility found was swede, Brussels sprout, hungry gap kale, cauliflower, rape, marrow stem kale, Savoy cabbage. Where Mo was omitted, yields were generally less than 10% of the Mo-sufficient plants. Interactions of Mo/Mn and source of N were also examined, using cauliflower and tomato. High Mo accentuated manganese toxicity. Leaf exudates from plants grown with excess molybdenum contained 4 p.p.m. of the element. The *Aspergillus niger* method has been used successfully to determine the Mo content of purified batches of sand, water and reagents in molybdenum deficiency experiments. Co-precipitation with cupric sulphide has been found to remove 99.9% of the trace amounts of molybdenum present in the chemicals. Pyrex glass containers have proved satisfactory for molybdenum studies. A survey has been made of the Mo content of a number of crop plants growing under normal and deficient conditions of molybdenum nutrition. Under the former, values obtained were (as p.p.m.): rye grass 1.3, cocksfoot 1.6, Yorkshire fog 4.2, alsike clover 5.6, lucerne 5.9, celery 6.3. Deficient plants contained from 0.05-0.1 p.p.m. Mo: cauliflower given excess Mo accumulated 413 p.p.m. Mo in the dry matter of the older leaves.

In laboratory experiments on tissue test techniques various macerates have been used for comparison and for specific purposes. Several solvents have also been tested as extractants.

The *Aspergillus* method for determining the availability of trace elements in soils has been further studied in experiments involving the examination of nitrate, nitrite and amino acids in the leaves of normal and Mo deficient plants. Mo deficient plants show in the leaves high nitrate, no nitrite, and low amounts of amino acids.

The amino acids of germinating seeds and of seedling cauliflowers have been examined at these stages. Observations were also made on the character and amounts of organic acids in the tissues.

Zinc deficiency in swede, tomato, beet and clover resulted in accumulation of glutamine and/or asparagine.

In pot experiments with barley and tomato, using P32 at levels of 0.5, 5, 25, 100 and 400 microcuries per pot, radiation effects were observed on the ratios fertiliser P uptake/total P uptake, fertiliser P uptake/soil P uptake, fertiliser P in tops/fertiliser P in roots. All effects were small and were judged to be insufficient to preclude the use of the method in plant nutrition studies provided good statistical designs are used.

In field trials with oats to determine the effects on yields of manganese sulphate and sulphur applied in various ways and the relationship of manganese content of plant organs to yields, sulphur gave erratic results whereas

manganese sulphate, either placed with the seeds or used at an early stage as a foliage spray, was consistent in its effects. Good correlation was found between the manganese content of mid-stem leaves and the levels of manganese treatment in the early stages of growth but not at later stages.

Results obtained on the acid soil plots from various liming and fertiliser treatments confirmed previous years' results as regards effects on the status of Mg, B, Mo, Mn and Al. Raising the pH by calcium and magnesian limestones eliminated all deficiencies and excesses present under the natural acid conditions but induced boron deficiency in sugar beet.

Whiptail in cauliflower was again successfully controlled on acid soils by lime and sodium molybdate respectively.

Pomology. Trials with the apple rootstocks originally selected at Long Ashton have been completed. Rootstock G8 appears suitable for standard trees.

Rootstocks MI and MII, from commercial sources, are being tested for latent virus infection. Tests are in progress to compare planting systems for apple trees, including cordons, dwarf pyramids and bush trees with and without fillers. A "hedge" system of planting apples is being used in tests of insecticides and fungicides.

Tests have been made of the viability of pollen of cider varieties in relation to compatibility problems. Records of blossoming dates and of leaf characters have also been continued. Sites have been selected for establishing 14 trial plots, 8 for bush trees and 6 for standards.

The effects of naphthalene acetic acid (NAA) on fruit set and fruit drop have been examined on a number of apple and pear varieties. Applied at full blossom, NAA reduced fruit set by interfering with pollen tube growth. Post-blossom applications temporarily inhibited but later increased fruit drop. These latter effects were accompanied by increased seed abortion in the fruitlets.

In pears (Conference and Williams) post-blossom applications of NAA increased the numbers of fruits harvested by as much as 100%.

Several naphthoxy aliphatic acids have been synthesised for growth promoting tests, including mono-, di- and tri-chloro naphth-oxyacetic acids. The effects of substitutions in the naphthoxy propionic acids have been examined and the resolution of 2-(3 chloro-naphth-2-oxy)propionic acid has been achieved by cinchonine. The *l*-form was completely without growth promoting activity, whereas both the *dl*- and *d*-forms showed activity.

The possible role of growth inhibitors and growth promoters in problems of dormancy of seeds and buds is under investigation.

Freshly harvested apple seeds contained an acidic substance which inhibits the growth of *Avena* coleoptiles. Maleic hydrazide appeared to delay the opening of black currant buds. Attempts to show the presence of auxins in dormant and non-dormant buds of black currant were unsuccessful.

The pollen of pear cultured *in vitro* needed the addition of 10-100 p.p.m. of boric acid to induce germination of the pollen grains.

Boric acid applied to ten varieties of pear in full bloom did not affect fruit set.

Pests and Diseases and their Control. In joint chemical and biological work on insecticides, the relation between molecular structure and toxicity has been explored for a number of organo-phosphorus materials and a range of DDT analogues. The effect of heat treatment in reducing the insecticidal activity of parathion has been demonstrated.

Further field trials have confirmed the effectiveness of a benzol-DDT-sulphite lye emulsion in controlling *Aphis pomi* on apples. The emulsion at

a strength of 0.05 DDT must be applied at the bud burst stage, when the aphids have hatched but are still on the outside of the buds.

The systemic insecticidal action of organo-phosphorus compounds has been further studied by experiments on the duration of activity of these compounds when added to soil and by investigating their rate of translocation in plant tissues.

The occurrence of systemic fungicidal action of certain phenoxy aliphatic acids has now been demonstrated and a method of screening materials for this property has been solved. Bean seedlings are grown with their roots in a 10 p.p.m. solution of the material to be tested, the bean leaves are then inoculated with *Botrytis fabae* and the subsequent lesion development measured. Several of the more highly chlorinated phenoxy acids have reduced the lesion growth rate by approximately 30 per cent.

In field trials of possible substitutes for lime sulphur in apple and pear scab control favourable results were obtained with glyoxalidines and a phthalimide compound. Eradicant fungicides have been tested in experiments on the control of brown rot and of apple canker.

A survey of virus diseases of stone fruit was made and experiments set up to determine the distribution of latent viruses in commercial apple stocks.

In the study of spray application, detailed assays have been made of the behaviour of plant protection materials applied by various types of equipment. Machines for the application of concentrate sprays have been designed, and have now been widely adopted for use in the Colonial Empire. Successful control of potato blight was obtained using a concentrated copper oxychloride spray at the rate of 10 gallons per acre.

Cider. The maturity of fruit at milling time in relation to the quality of the cider was studied, using the variety Dabinett. The results suggest that the optimum period of maturity is relatively long.

The processes of maceration and defecation of juices have been examined on four varieties with widely different juice characters. The treatments increased the control of fermentation. Maceration increased the pectin content of the juice and defecation lowered the content of both nitrogen and pectin. The effects of naturally occurring yeasts on the nitrogen and phosphorus constituents of juices have been followed during fermentation. Approximately 66% of the soluble N and 63% of soluble P were utilised during this process.

Various methods of controlling fermentation rates have been tested including the addition of yeast nutrients and stabilising factors such as centrifuging.

Eight selected yeasts have been used to ferment juices to compare their effects on quality against natural yeasts. The tests were made along with detailed biochemical and microbiological investigations. The ciders from the selected yeasts were judged to be of high quality.

Detailed chemical investigations were carried out on methods of alcohol determination and on buffer systems in relation to acidity changes during the course of fermentation.

Chromatographic methods have been applied to the determination of sugars, acids and phenolic compounds in juices and ciders.

In microbiological investigations yeast characters have been examined and detailed studies made of the organisms causing "cider sickness." The isolation and nutrition of the organisms have received special attention. It is proposed that the various strains of the organism shall be grouped under the name of *Saccharamonas pomonacea*.

The molybdenum content of juices was increased by branch injections of sodium molybdate.

Special attention is being given to metallic contamination and natural contents of metals in cider and juices in view of pending regulations.

Fruit Juices. The effects of long storage of fruits on subsequent juice quality have been followed on a number of varieties. Storage resulted in decreases in acidity, specific gravity and tannin content, and an increase in reducing sugars. Pectin showed an initial rise followed by a fall.

Ion exchange methods have been used in tests to de-acidify and demineralise juices, and to determine their efficacy for removing metallic contamination.

It has been shown that the pectase of apples, although low in amount, is sufficient to produce relatively rapid changes in pectin solutions and juices. The full results of this investigation have been published. Experiments have also been made on pH effects on pectase activity and the temperature conditions necessary to inactivate the enzyme in apple juice.

It was confirmed that the ascorbic acid content of black currants is reduced by nitrogenous manuring though the yield of ascorbic acid per acre may be increased.

Domestic Preservation of Fruit and Vegetables. Heat penetration tests on bottled and canned fruits have confirmed previous results and shown the need for revising times and temperatures of heating to obtain efficient sterilization.

Further tests on the "browning" of pears, plums, apples and peaches have confirmed that relatively high temperatures are needed to inactivate the enzymes responsible for the condition.

Preliminary experiments have been made on "deep freeze" methods and the possibility of using an ice-cream storage cabinet for the preparation of frozen products.

The method of microbiological assay employing *Lactobacillus helveticus* used for determination of vitamins in the B₂ group, has been adapted for the estimation of some of the micro-nutrient elements.

Other Activities.

Members' Day. The annual field day for members and the Annual General Meetings of Members and Governors of the National Fruit and Cider Institute were held on July 11th, 1950.

A programme illustrating important investigations in progress and practical aspects of fruit growing had been prepared. The main items demonstrated were as follows:—

1. *Field plots*—black currant and strawberry manurial trials; rootstocks for pear varieties; mineral deficiency effects on apples and potatoes; planting systems for apples; soil management in orchards; machinery for pest and disease control.

2. *Pot culture exhibits*—mineral balance in manuring potatoes, iron/phosphorus relationships; equipment and techniques for sand culture experiments.

3. *Cider house exhibits*—cider varieties recommended for planting; crop records from bush and standard orchards; distribution of cider orchards in the south-west; methods used in apple juice manufacture.

4. *Laboratory exhibits*—methods for the diagnosis of mineral status of crops—chemical tissue tests and the *Aspergillus* technique for trace elements; radioactive “tracer” methods; plant hormones for control of fruit drop; blossom thinning sprays; systemic insecticides; virus diseases of tree fruits; retention of vitamin C in jams.

Cider Sampling Day. The annual sampling day was held on May 4th. The function was similar in character to those held from 1946 onwards. During the morning session four groups of ciders were sampled. Group I consisted of ciders illustrating the main features of the 1949 season's crop. The point of interest in these ciders was that in spite of the exceptionally warm and sunny summer of 1949, the ciders were not of high vintage quality. This was probably due to the fact that the fruit developed abnormally late in the season under the influence of autumn rains. Group II comprised ciders made by the use of selected yeasts for comparison with ciders fermented in the usual way. Opinion was generally in favour of those made with selected yeasts. Group III illustrated the qualities of ciders which result from maceration and defecation (keeving) treatments of the juices. These ciders were of special interest in view of the widespread use of the practices in the centres in France to be visited subsequently during the 1950 tour of the National Association of Cider Makers. Defecation is also of interest at present as a possible method of conserving the natural sugars in cider. Group IV included ciders made from commercial apple varieties and a matured perry of high quality from the variety Blakeney Red.

In connection with the sampling function, exhibits of work in progress in the cider and fruit juices sections were shown in the cider house by means of charts and specimens. During the afternoon demonstrations of points of management of bush cider plantations were given.

Visitors. As in the previous year fifty-three organised parties visited the Station, this being the maximum number that could be received. The parties included a group of N.A.A.S. Horticultural Officers who attended for a two-day course, a French Delegation on cider making, Ministry of Education Housecraft Teachers, a party of Horticultural Instructors from Eire, Records of the National Institute of Agricultural Botany, the Fertiliser Society, the Society of General Microbiology and the Royal Institute of Chartered Surveyors. The full list is as follows :—

- Association of Scientific Workers (Bristol Group).
- Association of Scientific Workers (North Gloucestershire Group).
- Association of University Teachers.
- Bath Domestic College Science Students.
- Bristol Aeroplane Company Junior Welfare Organisation.
- Bristol and District Licensed Victuallers Association (2 parties).
- Bristol Class Teachers' Association.
- Bristol College of Technology Pharmacy Students' Association.
- Bristol 20-40 Club.
- Bristol University Mathematical Society.
- Bristol University Microbiological Society.
- Bristol University Chemical Society.
- Burderop Park Training College, Wroughton, Wilts.
- Cardiff University College Bacteriology and Botany Students.
- Chudleigh Allotment and Garden Association.
- Clifton College Scientific Society.
- Clifton High School Club, Bedminster, Bristol.
- Colston's Girls' School, Bristol.
- Dauntsey's School, West Lavington, Wilts.
- Devon Farm Institute Horticulture Students.
- Ditcheat Youth Club, Somerset.
- Dorset Growers.
- Eire Horticultural Instructors.

Fishponds Diocesan Training College, Barrow Court, Somerset (2 parties).
 French Delegation on Cider Making.
 Hallen and Berkeley Young Farmers' Club.
 Henleaze Townswomen's Guild, Bristol.
 Herefordshire Gardeners' Association.
 Ilminster Young Farmers' Club, Somerset.
 Kewstoke Women's Institute Produce Guild, Somerset.
 Long Ashton School.
 Lulsgate Volunteer Agricultural Camp, Somerset.
 Ministry of Education Canteen Management Course.
 Ministry of Education Housecraft Teachers' Course Students.
 National Agricultural Advisory Service (Refresher Course).
 National Institute of Agricultural Botany Recorders.
 Newport and District Horticultural Society, Monmouthshire.
 Pest Control Ltd.
 Royal Institute of Chartered Surveyors.
 Society for General Microbiology.
 Somerset Farm Institute Students (2 parties).
 Stapleton Group Townswomen's Guild, Bristol.
 St. Paul's Men's Training College, Cheltenham.
 The Fertiliser Society.
 Thornbury Young Farmers' Club, Gloucestershire.
 University College of Wales (Aberystwyth and Bangor).
 Wedmore Young Farmers' Club, Somerset.
 W. D. & H. O. Wills, No. 1 Factory.
 Worcester Training College.
 Yorkshire Young Farmers' Club.

Many scientists from overseas countries visited the Station, a number of whom worked in the laboratories for short periods to study special methods or particular subjects. Among these visitors the following may be specially mentioned.

Visitors from Overseas (1950).

- Argentina :* Mr. T. C. S. Haslam, Fruit Grower.
Australia : Dr. I. Clunies Ross, Commonwealth Scientific and Industrial Research Organisation.
 Mr. R. D. Wilson, Head of Entomology Section, C.S.I.R.O., New South Wales.
 Mr. J. K. Taylor, Director of Soil Survey for Australia, C.S.I.R.O., Waite Institute, Adelaide.
 Mr. A. L. C. Davidson, Division of Soils, C.S.I.R.O., Waite Institute, Adelaide.
 Mr. A. J. Anderson, Senior Research Officer, C.S.I.R.O., Canberra.
Belgium : Dr. D. Stenuit, Director, Service Pedologique de Belgique, Louvain.
 Mons. A. Witters, Service Pedologique de Belgique, Louvain.
 Mons. R. L. Piot, Service Pedologique de Belgique, Louvain.
Brazil : Prof. A. Chaves Batista, Head of Phytopathology Division, Instituto de Pesquisas Agronomicas, Recife.
Canada : Mr. W. N. Keenan, Plant Quarantine Laboratory.
 Dr. L. W. Koch, Harrow Laboratory.
 Prof. L. P. V. Johnson, Division of Genetics and Plant Breeding, University of Alberta.
 Dr. W. J. Hagborg, Dominion Laboratory of Plant Pathology, Winnipeg.
 Dr. J. W. Groves, Mycologist, Ottawa.
 Mr. H. L. Seamans, Chief, Division of Entomology, Ottawa.
Chile : Dr. Manoz.
 Sr. Elias Letelier, Research Chemist, Ministry of Agriculture.
Colombia : Mr. J. G. Hawkes, Bogota.
Denmark : Mr. Ivar Hellborn, Messers. Beauvais A/S, Copenhagen.
 Mr. Jeppeson, Fruit Grower.
East Africa : Mr. Kenneth S. McInlay, Entomologist, East African Agriculture and Forestry Organisation.
Egypt : Mr. M. El Beheiri, Assistant Chief Entomologist, Royal Egyptian Agricultural Society.
France : Prof. P. Boischet, Director, Central Station of Agronomy, Versailles.
 Prof. R. Chaminade, Ministry of Agriculture Horticultural Research Station, Versailles.
 Mons. Y. Coie, Director, Laboratory of Vegetable Physiology, Versailles.

- Germany :* Miss Bernardine Hardt, University of Bonn.
Gold Coast : Mr. P. H. Nye, Government Agricultural Chemist.
India : Mr. N. G. Gokhale, Tocklai Experimental Station, Assam.
Israel : Mr. M. Winnik, C.E., Director of the Soil & Fertiliser Research Institute, Mikveh-Israel.
 Dr. Lipovetzky.
Jamaica : Mr. Egbert A. Tai, Senior Agricultural Officer, Department of Agriculture.
Malaya : Mr. Lever, Entomologist.
 Dr. C. E. T. Mann, Director, Rubber Research Institute.
Mauritius : Dr. Halais.
New Zealand : Mr. L. Morrison, Canterbury Agriculture College, Lincoln.
 Mr. F. R. Callaghan, Secretary, Department of Scientific and Industrial Research, Wellington.
 Prof. V. J. Chapman, Professor of Botany, Auckland University College.
Nigeria : Mr. G. E. O. Okiy.
 Dr. Ir. K. Ebes, Lecturer in Tropical Agriculture, University College of Nigeria, Ibadan.
South Africa : Mr. Owen Jones, I.C.I.
 Dr. W. S. Martin, Wattle Research Institute, University of Natal, Pietermaritzburg.
 Dr. A. C. Myburgh, Western Province Fruit Research Station, Stellenbosch.
 Dr. T. D. Hall, African Explosives & Chemical Industries Ltd.
 Mr. S. J. Cillie, Engineer, Western Province Fruit Station, Stellenbosch.
Southern Rhodesia : Mr. Keith Sturgess.
 Mr. C. N. Hayter, Government Horticulturalist.
Spain : Dr. R. Dios, Highest Research Council.
 Prof. C. Rodriguez, University of Zaragoza.
Sweden : Mr. C. Castberg, Nynashamn.
 Dr. Nils Karlsson, Royal Agricultural College.
Trinidad : Mr. J. S. Campbell, Agricultural Chemist, Imperial College of Tropical Agriculture.
 Prof. F. Hardy, Imperial College of Tropical Agriculture.
 Mr. E. W. Simmonds, Imperial College of Tropical Agriculture.
 Dr. B. G. Montserin, Cocoa Agronomist.
U.S.A. Dr. R. Q. Parks, Division of Soil Management and Irrigation, U.S. Dept. of Agriculture, Beltsville, Maryland.
 Dr. H. C. Knoblauch, Assistant Chief, Agricultural Research Administration, Washington, D.C.
 Dr. J. Einset, Assistant Professor of Pomology, Geneva Agricultural Experiment Station, New York.

It was a pleasure for members of the staff to meet so many colleagues from overseas and to discuss with them problems of mutual interest.

Shows and Demonstrations. Exhibits were staged at the following shows :

- Bath and West Show*—Birmingham, May 31st to June 3rd.
Brewers' Exhibition—London, October 2nd to 6th.
Bristol Horticultural Show—Bristol, August 30th to September 1st.
Royal Agricultural Show—Oxford, July 4th to 7th.
Royal Horticultural Society—Chelsea, March 23rd to 26th.
Royal Horticultural Society—London. Fortnightly Shows. March 21st and October 10th.
Three Counties Show—Worcester, June 13th to 15th.

Students and Voluntary Workers. The number of research students must still remain severely limited owing to lack of bench space. This is most unfortunate since, during the post-war period, numerous applications have been received from graduates wishing to pursue post-graduate studies at the Institute. The list of applicants has included some eminent senior workers from abroad who would have liked to spend periods in our laboratories. It is also regretted that the accommodation we have been able to offer to those who have worked with us has been so limited, often entailing inconvenience and discomfort.

Dr. R. J. W. Byrde completed his work on Brown Rot of apple and plum, under Mr. Marsh, by arrangement with Professor Stoughton and Reading University and, as reported in an earlier section, was later appointed to the Institute staff.

Dr. S. C. Agarwala, Lecturer in the Botany Department, Lucknow University, who is the holder of a United Provinces (India) research studentship, is spending two years at the Institute in the plant nutrition section, under Dr. Hewitt, working on problems of the molybdenum nutrition of crops. He commenced his studies on 6/5/50 and is registered as a Ph.D. student in the University.

Mr. E. J. Skerrett has continued his work in organic chemistry under Dr. Woodcock and is now registered as a Ph.D. student in the University.

Monsieur Robert Cardinaud, l'Ecole Nationale Supérieure Agronomique, Toulouse, spent the period 17/7/50 to 2/9/50 in the Station plantations attached to the crop recording staff.

Miss D. M. Payne spent three months as a voluntary worker in entomology and photography under Dr. Kearns.

The following also spent short periods working in the laboratories or plantations : Mr. M. W. H. Coloham, student of Lincoln College, Oxford, in the plant nutrition section with Dr. Hewitt and Dr. Nicholas ; Mr. P. Hollier, of Messrs. Unilever—special course on diagnostic methods for determining the mineral status of crops—also in the plant nutrition section ; Mr. J. J. Jeffery, student of University College, Southampton, as a voluntary worker in Entomology under Dr. Kearns ; Mr. K. S. McKinley, Entomologist in the East African Agriculture and Forestry Research Organisation, at the request of the Colonial Office, to study spraying machinery under Dr. Kearns ; Mr. Erik Johnsen, M.Sc., Statens Hagebruksskule po Hjeltnes, Ulvik, Norway, to study problems of spray damage ; Mr. Jens Wellejus, Svendborg, Denmark, as a voluntary worker in the plantations to gain experience in pruning methods ; Miss E. Knulenu, Gold Coast, spent ten weeks in the Fruit Preservation section under Miss Crang, at the request of the Colonial Office, studying domestic methods of preservation. She also took one of the courses for instructresses and obtained a certificate.

Mr. Roy Abbott completed his period of training in cider making, as holder of the Memorial Studentship, on March 31st, and his successor, Mr. J. H. Evans, began his course on 1/10/50.

Mr. J. Pullin, Compton Greenfield, and Mr. A. Pullin, Falfield, have both registered for courses in cider making. The former commenced work on 2/10/50 and the latter on 16/10/50.

The following courses were given in the Domestic Fruit and Vegetable Preservation Section :—

Preservation and Judging Preserves—July 10th–28th.

Preservation of Fruit and Vegetables—Aug. 14th–25th.

Preservation of Fruit and Vegetables—Aug. 28th–Sept. 8th.

Thanks are due to Miss Margaret Spinks for assistance given in the courses from August 14th to September 8th.

A course on fruit canning was given at the request of the Women's Voluntary Services from April 18th–21st, attended by their members from the different regions in England, Scotland and Wales.

Social Activities. The Welfare Committee, under the chairmanship of Dr. Woodcock, has continued to look after this side of the Station's activities.

The Tennis Club has been very active and carried through a programme of matches in the Bristol Tennis League. Both the hard and grass courts were in use throughout the season.

The Cricket Club has continued to run under serious disadvantages, even

more so than in 1949, as the Long Ashton Cricket Club found it necessary to curtail the facilities offered to the Station Club for home matches in previous seasons. Fixtures were thus mainly restricted to "away" matches but the club also entered the tournament for the Evening Post Challenge Cup.

The annual Christmas staff party was again held in the Village Hall on December 20th, when over 260 attended. The staff were pleased to welcome Capt. and Mrs. Wills and Mr. Woodall on this occasion. The main event of the evening's entertainment was a play written, produced and performed by members of the staff.

Acknowledgments. Special acknowledgments are due to members of the staff who have carried out special duties in addition to their normal work. Dr. Kearns has continued to give valuable assistance in connection with the planning of new buildings and in organising and supervising the workshops, including repair work to equipment, machinery and tools for the laboratories and farm. Mr. Spinks continued to act as librarian and was responsible for editing the 1949 Annual Report in the absence of Mr. Marsh. Dr. Woodcock continued in the office of Chairman of the Welfare Committee and was responsible in this capacity for organising the Christmas Social. Miss Sturdy again acted as Chairman of the Canteen Committee. Best thanks are due to these and to other members of staff who have helped in various ways during the year.

Grateful acknowledgment is also made to all members of the assistant and technical staffs and to all employees for their loyal support in carrying out the programme of research work.

Special thanks are also due to Mr. J. E. Beviss who gave valuable assistance during the early summer in the distribution of budding material.

Thanks are also due to all outside friends, particularly to growers and commercial firms, who have co-operated in experiments or helped in other ways during the year.

A PRELIMINARY INVESTIGATION INTO THE NATURE OF THE HORMONE PRODUCED BY DEVELOPING APPLE SEEDS.

By L. C. LUCKWILL and D. WOODCOCK.

Introduction.

Since the discovery at Long Ashton in 1946 that apple seeds at certain stages of development produce a natural phytohormone (5), investigations have been in progress to determine the possible part played by this hormone in the development of the apple.

Early experiments showed that the presence of the hormone could be detected by the ability of aqueous extracts of apple seeds to induce parthenocarpy in tomatoes, and a quantitative assay method based on this reaction was developed (6). Later it was shown that the hormone was also active in delaying the abscission of Ivy petioles from which the lamina had been removed. Investigations in 1947 with the apple variety Beauty of Bath, in which the hormone content of the seeds was measured at weekly intervals from petal-fall till harvest suggested a relationship between the production of this hormone and fruit drop (7), and this has since been confirmed in similar experiments with Lane's Prince Albert and Crawley Beauty carried out in 1948 and 1949. These experiments have shown that during the development of the seed there are two peak periods of very active hormone production. The first of these occurs three to four weeks after petal-fall and marks the end of the first wave of fruit drop, whereas the second and major peak, eight to ten weeks after petal-fall, coincides with the cessation of the "June" drop. It has been further established that the endosperm tissue of the seed is the probable source of this hormone and that the fluctuations in hormone content which occur throughout the season are associated with definite stages in endosperm development.

In addition to these experiments, which have been reported upon elsewhere (7, 8), a number of tests have been carried out to compare the efficiency of various methods of extracting the hormone from seeds, and to establish some of its basic chemical and physical properties. The results of these tests are reported below :—

Experimental Data.

Standard extraction method.

The following method of preparing active hormone extracts from apple seeds has been used throughout the experiments on fruit drop, and is referred to here as the standard method :

Apple seeds, immediately on removal from the fruit are dried in an oven at 100–120° C. and ground to a fine powder with a pestle and mortar. A known weight of this seed powder is then boiled in an open beaker with a minimum quantity of water for 15 minutes, and whilst still hot, the extract is filtered through glass wool. The residue is reboiled for a further 15 minutes and is again filtered. The combined water extracts, after cooling and filtering to remove the precipitate of phloridzin, are then shaken four times with approximately five times their own volume of ether (peroxide free). The ether layers are separated, dehydrated over anhydrous sodium sulphate and evaporated

to dryness in a small pyrex test tube. The dry residue is then dissolved in 1 ml. distilled water and from this solution a series of five dilutions, corresponding to 1, 1/3rd, 1/10th, 1/30th and 1/100th of the original extract is prepared for testing.

Hormone assay.

The assay method used has been described in detail elsewhere (6) and only a summary of the technique will be given here. The method depends on the ability of the active substance to stimulate parthenocarp in the tomato. A known volume of extract (0.0225 ml.) is added to each of a series of 8 or 10 unpollinated tomato ovaries, and after a period of six days the diameters of the young fruitlets are measured. By comparing the growth of the ovaries with that induced by known quantities of a synthetic growth substance the relative activity of different extracts can be compared. The standard growth substance used on the controls throughout these experiments was 2-naphthoxy-acetic acid (2-NOA), and the hormone yields throughout have been expressed as $\mu\text{g.}$ equivalents of 2-NOA per 100 gm. dry weight of seed.

This assay method has given very satisfactory results, but on account of the large number of tomato plants required (20 per extract), it has been found possible to test only a limited number of extracts during the course of a season.

Acid fractionation.

In order to determine whether the active substance was acid or neutral in reaction a number of acid fractionations were carried out, the results of which are summarised in Table I.

Extracts 100 and 152 were prepared by the standard procedure outlined above. In extracts 108 and 159 the acidic substances were removed from ether solution by shaking with saturated sodium bicarbonate. After separation, the aqueous phase was acidified and again shaken with ether. The acid fraction now passed into the ether layer and was separated, dried and taken up in water for testing. The neutral fraction, recovered from the original ether solution, was treated in the same way.

Extracts 188 and 201 were fractionated according to the method of Boysen Jensen (2) in which the aqueous phase is saturated with glucose in order to increase the efficiency of the ether/water separation.

TABLE I.
ACID FRACTIONATION OF APPLE SEED EXTRACTS.

Variety	Date of Collection	Extract number	Fraction	Hormone yield $\mu\text{g.}$ 2-NOA per 100 gm.
Miller's Seedling	26/6/47	100	Not fractionated	1770
		108a	Neutral	0
		108b	Acidic	580
Miller's Seedling	10/6/48	152	Not fractionated	1260
		159a	Neutral	0
		159b	Acidic	950
Miller's Seedling	25/7/49	188a	Neutral	0
		188b	Acidic	190
Crawley Beauty	12/8/49	191	Not fractionated	23
		201	Acidic	20

It is clear from Table I that the active hormone must be acidic in nature. No evidence of hormone activity has been found in the neutral fractions. The apparent low recovery of hormone in the acid fraction of extract 108 is probably due to excess acid which caused some damage to the test ovaries. In subsequent fractionations the acidity of the final extracts was adjusted more carefully and, where the glucose method was used (Extract 201), 86% of the hormone originally present was recovered in the acidic fraction.

Stability to acids and alkalis.

Kögl, Haagen-Smit and Erxleben (4) have shown that pure auxin A retains its activity after heating with strong acid, but is destroyed by strong alkali. In this respect it differs from auxin B, which is destroyed by both acid and alkali hydrolysis, and from indolyl acetic acid which is stable to alkali but not to acid.

In order to test the acid and alkali stability of the hormone from apple seeds two experiments have been carried out, the results of which are shown in Table II. In both these experiments the original extracts were made by the standard method. After dehydration the ether solution was divided into four equal portions, each of which was taken to dryness in a 25 ml. conical flask. To one flask was added 5 ml. 1N.HCl; to another 5 ml. 1N.NaOH; and to a third 5 ml. distilled water. These three flasks were then autoclaved for four hours at 15 lb. pressure. After adjustment of the pH until just acid, the contents of each flask were re-extracted with ether to recover any active substance which remained. The extract in the fourth flask, which was not autoclaved, was taken up in water and tested directly.

TABLE II.
ACID AND ALKALI STABILITY OF THE HORMONE FROM APPLE SEEDS.

Variety	Date of Collection	Extract number	Treatment	Hormone yield μ g. 2-NOA per 100 gm.
Crawley Beauty	1/7/48	160a	Autoclaved 1N.HCl	0
		160b	" 1N.NaOH	0
		160c	" water	397
		160d	Not autoclaved	500
Miller's Seedling	10/6/48	163a	Autoclaved 2N.HCl	0
		163b	" 2N.KOH	0
		163c	" water	active*
		163d	Not autoclaved	active*

* Owing to the small number of test plants available and the low activity of these extracts, quantitative assay was not possible.

The results of these experiments show that the hormone is both acid-labile and alkali-labile, but that it is not destroyed by autoclaving with water for four hours. In this respect the properties of this hormone agree with those of auxin B, but are inconsistent with those described for auxin A or indolyl-acetic acid.

Solubility in petrol-ether.

For extract 192a the standard procedure of boiling in water and extracting with ether was used. 192b was similar except that the seeds were first treated for half-an-hour with petrol-ether (B.P. 40–60° C.) in a Soxhlet extractor.

In 192c the extraction was carried out in the usual way, but before shaking with ether the water extract was washed for 5 minutes with three times its own volume of petrol-ether.

As can be seen from Table III treatment with petrol-ether, both before and after water extraction, reduced the hormone yield, suggesting that the active substance is soluble to some extent in this solvent.

TABLE III.
SOLUBILITY OF APPLE SEED HORMONE IN 40/60 PETROL-ETHER.

Variety and date of collection	Extract number	Treatment with petrol-ether	Hormone yield $\mu\text{g. equivs.}$	% loss
Crawley Beauty 12/7/49 to 28/7/49	192a	None	726	—
	192b	Dry seeds pre-extracted with petrol-ether	650	10.5
	192c	Water extract washed with petrol-ether	500	31.0

Efficiency of the standard extraction method.

The "standard" method of preparing active extracts is not claimed as an exhaustive extraction, though, when conducted under standard conditions, it might be expected to remove a constant proportion of the total hormone present. In order to obtain some idea of the number of water/ether extractions necessary for complete removal of the hormone the following experiment was carried out:—

3.5 gm. of dried and powered seeds (Miller's Seedling, 16/7/47) were boiled with 35 ml. water for 15 minutes. After filtering through glass wool the residue was re-boiled with 25 ml. water for a further 15 minutes. Two further 15 minute extractions were carried out on the same sample of seed, and each of the four water extracts was shaken with five separate lots of 100 ml. ether. The total quantity of hormone in each of the 20 ether washings was then assayed. (Table IV).

TABLE IV.
QUANTITY OF HORMONE ($\mu\text{g. equivs. 2-NOA}$)
REMOVED FROM 3.5 gm. SEEDS BY SUCCESSIVE 15-MINUTE EXTRACTIONS WITH BOILING WATER.

Water extractions	Ether washings					Total
	1st	2nd	3rd	4th	5th	
1st	10.2	5.8	4.0	3.1	2.2	25.3
2nd	9.8	3.6	2.7	2.2	2.7	21.0
3rd	6.2	2.7	2.2	1.8	0	12.9
4th	4.0	2.7	2.2	1.8	1.7	12.4
Total	30.2	14.8	11.1	8.9	6.6	71.6

Table IV shows that even after boiling with three successive lots of water for 15 minutes each, appreciable quantities of hormone still remain in the seed, and it seems likely that at least five or six such extractions would be necessary for complete removal of the active substance. Each water extract needs to be shaken with ether at least five or six times to remove all the hormone from aqueous solution. It is estimated from this experiment that the standard extraction method described on page 23 removes not more than 50 to 60% of the total hormone present in the seeds.

Extraction with ether.

In view of the difficulty experienced in the complete removal of hormone from the seed with boiling water, extraction with ether was tried. The hormone yields obtained by this method were compared with those obtained by water extraction in a series of experiments, the results of which are tabulated in Table V.

TABLE V.
COMPARISON OF HORMONE YIELDS
OBTAINED FROM DRIED APPLE SEEDS BY WATER AND ETHER EXTRACTIONS.

Variety and date	Extract number	Method of Extraction	Hormone yield $\mu\text{g. equivs. per}$ 100 gm.
Laxton's Superb 1/6/48	158a	Satd. aq. ether—3 hours—Soxhlet extraction	475
	158d	Dry ether—3 hours—Soxhlet extraction	450
		Same seeds re-extracted boiling water 3×15 mins.	424
	158b	Boiling water— 3×15 mins.	816
	153	Boiling water—1 hour under reflux	696
Beauty of Bath 24/6/47	97	Dry ether—4 hours—Soxhlet extraction	740
	99	Boiling water— 2×15 mins.	2600
Laxton's Superb 7/7/48	162	Dry ether—3 hours—Soxhlet extraction	440
		Same seeds re-extracted boiling water 2×15 mins.	1590
Crawley Beauty 26/9/49	195	Dry ether—3 hours—Soxhlet extraction	533
		Dry ether—further 3 hours Soxhlet extraction	233
		Same seeds re-extracted boiling water 2×15 mins.	776
	194	Water—3 days at room temperature Same seeds re-extracted boiling water 2×15 mins.	1460 0
Crawley Beauty 4/8/50	228	Dry ether—5 hours—Soxhlet extraction	94
		Dry ether—further 5 hours Soxhlet extraction	56
		Dry ether—further 5 hours Soxhlet extraction	0
		Same seeds re-extracted boiling water 20 mins.	158
Crawley Beauty 4/8/50	229	Dry ether—12 hours—Soxhlet extraction	36
		Same seeds re-extracted cold water 20 hours	36

In general it was found that Soxhlet extraction of seeds with dry (or damp) ether for periods varying from 3 to 15 hours gave hormone yields which were only 30–50% of those obtained by two 15-minute boiling water extractions. This can be seen from a comparison of extracts Nos. 97 and 99, 158a, b and d.

Moreover, it was found that seeds, after prolonged extraction with ether, would still yield further substantial quantities of hormone if they were subsequently extracted with boiling water. (See extracts Nos. 158d, 195, 162 and 228). This was so even where the ether extraction was prolonged to 15 hours (extract 228), by which time all ether soluble material in the seeds had been removed.

In these experiments it was found that water at room temperature, as well as boiling water, could be used for extraction and that seeds which had already been extracted with ether for 12 hours would yield further hormone on soaking in cold water. (Extract 229). After soaking dried seeds in water at room temperature for three days no further hormone could be detected in the seeds. (Extract 194).

The fact that only part of the hormone present in the seeds is directly extractable with ether suggests that the remainder is present in some "bound" form, or as an inactive precursor, from which the active hormone is liberated by hydrolysis when the seeds are soaked or boiled in water.

The occurrence of auxin in "free" and "bound" form has already been shown for the endosperm of maize and other cereals, which yield considerable amounts of auxin when extracted with water but little or none when extracted with organic solvents (1). In the apple seed too, the endosperm appears to be the chief seat of hormone formation, though smaller amounts can also be extracted from the growing embryo (7). The experiment reported in Table VI in which endosperms and embryos were extracted separately, first with ether and then with boiling water shows that the ratio of precursor/free hormone is much greater in the endosperm than in the embryo.

The fact already noted (see Table III) that petrol ether removes only small amounts of hormone from dry seeds and much greater amounts from water extracts can now be explained, if it be assumed that this solvent removes only "free" hormone and not inactive precursor. It is also clear from these experiments that the precursor must be insoluble in ether, otherwise it would be removed by prolonged Soxhlet extraction with this solvent (e.g. in extracts 195, 228 and 229).

TABLE VI.
YIELDS OF "FREE" (I.E. ETHER EXTRACTABLE) AND "BOUND" HORMONE IN EMBRYOS AND ENDOSPERMS OF LAXTON'S SUPERB (COLLECTED 7/7/48).

Part of seed	Method of extraction	Hormone yield μg. equivs. per 100 gm.
Embryo	Ether—Soxhlet extraction—3 hrs.	370
	Same material boiled in water 2 × 15 mins.	650
Endosperm	Ether—Soxhlet extraction—3 hrs.	510
	Same material boiled in water 2 × 15 mins.	2540

Avery et al. (1) have shown that the hydrolysis of precursor to free auxin in cereal endosperms is most effectively accomplished at pH 10. In

our experiments hydrolysis was always carried out at the natural pH of the extracts, which were invariably slightly acid. It is possible, therefore, that by raising the pH more rapid total extraction could be achieved.

In cereals the hormone released by hydrolysis of endosperm extracts has been identified as being chiefly indolyl acetic acid (3), though in wheat an alkali-labile auxin has also been identified. It is thought that the hormone from apple seeds cannot be indolyl acetic acid for the following reasons :—

- (1) It is highly active in the tomato parthenocarp test, whereas indolyl acetic acid is practically inactive.
- (2) Seed extracts have so far failed to show any response in tests based on cell elongation (e.g. *Avena* cylinder test; tomato leaf epinasty test). This evidence is not conclusive since the lack of response might be due to elongation-inhibiting substances present in the extract. Nevertheless, the indications are that the active substance is not an "auxin," in the usual sense in which this term is used for a substance promoting cell elongation.
- (3) Apple seed hormone appears to be alkali labile, whereas indolyl acetic acid is alkali-stable.

Loss of activity on keeping.

It has been frequently noted that samples of dried seeds kept in corked tubes in the laboratory gradually lose their activity over a period of one to two months. This loss of activity can be seen by comparing extracts 228 and 229 (Table V) which were made on the same batch of seeds. No. 228 made and tested 12 days after collection of the seeds gave a total yield of 308 μ g. equivs. per 100 gm., compared with only 72 μ g. from No. 229 which was made 14 days later.

Seed extracts, whether dry or in water also lose activity on keeping. Active extracts in water and lanolin were placed in storage at -15° C. in September, 1946, but had lost all activity when removed from cold storage in April, 1947.

Summary.

The following properties have been established for the active hormone which occurs in the endosperm, and to a lesser extent in the embryo, of the developing apple seed :—

The hormone is acidic in nature; it is stable to heat but is destroyed by hydrolysis with strong acid or alkali, and loses its activity on keeping for one to two months. It is soluble in ether and water, and apparently, to a limited extent, in petrol ether.

In seed which has been dried at $100-120^{\circ}$ C. it is present in two forms : "free" hormone which can be removed by ether extraction, and "bound" hormone or precursor, which readily yields free hormone when the seeds are placed in cold or hot water. In the seed samples investigated more than one half of the total hormone present was in the form of precursor. The precursor is insoluble in ether and petrol-ether.

From its chemical and physiological properties it is concluded that the hormone cannot be identical with indolyl-acetic acid.

REFERENCES.

- (1) AVERY, G. S., JR., BERGER, J. and SHALUCHA, B. (1941). The total extraction of free auxin and auxin precursor from plant tissue. *Am. Journ. Bot.* 28 : 596-607.

- (2) BOYSEN-JENSEN, P. (1941). Quantitative Bestimmung der beschleunigenden Streckungswuchsstoffe in der sauren Fraktion der Ätherextrakte aus höheren Pflanzen. *Planta* 31 : 653-669.
- (3) HAAGEN-SMIT, A. J., LEECH, W. D. and BERGREN, W. R. (1942). The estimation, isolation and identification of auxins in plant material. *Am. Journ. Bot.* 29 : 500-6.
- (4) KÖGL, F., HAAGEN-SMIT, A. J. and ERXLEBEN, H. (1934). Über den Einfluss der Auxine auf das Wurzelwachstum und über die chemische Natur des Auxins der Graskoleoptilen. *Hoppe-Seyl. Zeit. Physiol. Chemie.* 228 : 104-112.
- (5) LUCKWILL, L. C. (1946). A fruit-setting hormone from apple seeds. *Nature* 158 : 663.
- (6) LUCKWILL, L. C. (1948). A method for the quantitative estimation of growth substances based on the response of tomato ovaries to known amounts of 2-naphthoxy-acetic acid. *Journ. Hort. Sci.* 24 : 19-31.
- (7) LUCKWILL, L. C. (1948). The hormone content of the seed in relation to endosperm development and fruit drop in the apple. *Journ. Hort. Sci.* 24 : 32-44.
- (8) LUCKWILL, L. C. (1949). Fruit drop in the apple in relation to seed development. *Ann. Applied Biol.* 36 : 567-568.

REPORT ON A TRIAL OF A NEW METHOD FOR CONTROLLED POLLINATION.

By J. D. CUTHBERTSON and RITA M. STICKLEY.

Introduction.

A shortage of paper or muslin bags in Germany after the war led Schanderl to investigate alternatives to the orthodox methods for the protection of flower stigmas after controlled pollination has been carried out. In a recent paper (1) he describes the use of vaseline for this purpose. Working with apples, pears and cherries Schanderl first pollinated the flowers and then applied a drop of vaseline to the stigmas. This he found successfully protected them from foreign pollen whilst allowing the pollen already applied to germinate and to fertilise the ovules.

By adopting this method it was found possible to dispense with the use of muslin or paper bags. In addition, emasculation of the treated flowers was found to be necessary only in so far as it was convenient to remove some stamens in order to expose the stigmas more fully. To avoid the occurrence of natural pollination, controlled pollination must be carried out before the flowers open and in this condition it may happen that the stigmas have not yet become receptive. There is therefore a delay in pollen germination until the stigmas reach the right state of maturity. It was found that pollination followed by the application of vaseline in such circumstances did not prevent subsequent fertilisation.

The saving of labour and materials is the chief advantage claimed for the new method, but the injurious effects of an artificial bag climate are also avoided. In view of this and of the simplicity of the method it was felt desirable to carry out further tests in order to assess its suitability for both indoor and outdoor pollination work on fruit trees. Accordingly experiments on apples were undertaken in the Spring of 1950.

These experiments were designed to test the following :—

1. The degree of protection obtained by the application of vaseline.
2. The effect of vaseline on pollen germination and on the subsequent fruit set.
3. The effect on subsequent fruit set of the application of vaseline before the stigmas have become receptive.
4. The practical possibility of the use of vaseline in the field.

It was anticipated that any effect produced by avoiding an artificial bag climate would probably be more indirect than direct, but nevertheless, treatments were arranged on the assumption that a direct effect on fruit set was a possibility. Emasculation was also considered as a separate factor.

Experimental.

Preparation of Vaseline Cream.

Initial trials showed that vaseline by itself was difficult to manipulate under the cold conditions normally prevailing in early Spring, and various diluents were tried in order to render the vaseline less viscous. Olive oil was found to be satisfactory. When this is mixed with vaseline in approximately equal parts the viscosity of the vaseline is reduced and a cream is formed. In practice the proportion of olive oil to vaseline was varied, to suit weather conditions on the day of application.

Effect of Vaseline on Pollen Germination* "in Vitro."

The effect of the vaseline on pollen germination and also its protective effect were ascertained "in vitro." 10% sucrose/agar films were prepared on microscope coverslips and pollen was sown on these films, a proportion being covered with vaseline. In order to examine protective effect, vaseline was spread directly on the sucrose/agar film, the pollen grains then being sown on the vaseline. All the preparations were mounted in the same manner as for a hanging drop culture and incubated at 25° C.

By covering pollen with vaseline the germination capacity of the grains was reduced by 30%. It was apparent however, that the sucrose/agar medium on which the pollen grains were cultured did not provide the optimum conditions for germination, as an increase in germination percentage was obtained by sowing the same sample of pollen in a hanging drop culture containing the same percentage of sucrose.

Pollen grains sown on the vaseline did not germinate.

Effect of Vaseline Treatment on Fruit Set.

1. *Lord Lambourne* × *Miller's Seedling*.

Twelve five-year-old pot grown trees of the variety *Lord Lambourne* were chosen for the first experiment. These were brought in under glass in March and flowered early in April. Pollen from the variety *Miller's Seedling* was used for cross pollination.

Six treatments (A to F), were applied to each of the twelve trees, one truss reduced to four lateral flowers being selected for each treatment on each tree. Thus each treatment was applied to a total of 48 flowers. A description of these treatments is given in Table I.

TABLE I.
DESCRIPTION AND TIMING OF TREATMENTS.

	Treatment			Stage
A	Emasculated	No Vaseline applied	Truss enclosed in Bag	1
B	Not Emasculated	Vaseline applied	Truss not enclosed in Bag	1
C	"	"	Truss enclosed in Bag	1
D	Emasculated	"	"	1
E	"	"	"	2
F	"	"	"	3

Pollination in treatments A to D was carried out when the flowers had reached the "balloon" stage, that is one or two days before the petals opened. This was designated as Stage 1. The flowers for the two remaining treatments E and F were emasculated and enclosed in paper bags at Stage 1, but pollination and the application of vaseline was delayed for two days for treatment E (Stage 2), and for four days for treatment F (Stage 3). In all other respects these two treatments were identical with treatment D. Treatments D, E and F form a timed series and comparison of these treatments gives some indication of the effect of stigma maturity on subsequent fruit set. In choosing the flower trusses it proved impossible to avoid some slight variation in state

of maturity and thus in effect there was some variation in the timing of the three stages. At Stage 2 the petals were generally full open and at Stage 3 petal fall had commenced.

A simple instrument consisting of a bronze wire loop 1/10th-inch in diameter attached to a dissecting needle was used for the application of the vaseline. The enclosure in one drop of vaseline of the five stigmas present in the apple flower presented no real difficulty as the vaseline tended to draw the stigmas together.

The amount of suitable flower material available on the pot trees was limited and a disproportionately high number of spur trusses were used for the unbagged treatments. Under the conditions of pot culture the terminal trusses set far more readily than did spur trusses and this therefore rendered any direct comparison of the two techniques unreliable. For the same reason the possible effects of bagging could not be considered. The results obtained from treatments A, C, D, E and F are given in Table II.

TABLE II.

EXP. 1: POT GROWN TREES. VARIETY: LORD LAMBOURNE.
(RESULTS FROM BAGGED TREATMENTS ONLY).

	Treatment				
	A	C	D	E	F
Stage of Flower Development	1	1	1	2	3
Initial Set %	60	58	54	48	29
Final Set %	31	40	33	19	4
Seed Count %	30	33	30	17	4

These show that vaseline application at Stage 1 had little effect on fruit set or subsequent fruit development (A and D). The same also applies to the effect of emasculation (C and D). The results obtained from application at the two later stages of flower development show decreases, most marked in the case of treatment F. A seed count was made at the time of the second fruit count and the percentages given have been calculated on a theoretical maximum yield of 480 seeds per treatment, that is a maximum of 10 seeds for every flower pollinated. These percentages thus give an indication of the seed yield which was obtained.

2. *Sweet Alford* × *Dabinett*.

A single row of 12 fifteen-year-old established bush cider trees of the variety Sweet Alford was used for the second experiment. The treatments applied in Experiment 1 were replicated in this experiment. To minimise the effect of the position of the truss on the tree, only those trusses borne as terminals were selected for treatment. The results of this experiment are given in Table III. Pollen was obtained from the cider variety Dabinett and pollination was carried out on the 12th, 14th and 16th May for the three stages of flower development. The first count, made on June 1st, was prior to "June drop" and that made on June 24th, followed "June drop." A seed count was made on August 19th, and data from this have been expressed as a percentage of the total possible, as in Table II.

TABLE III.
EXPERIMENT 2: BUSH TREES. VARIETY: SWEET ALFORD.

	Treatments					
	A	B	C	D	E	F
Stage of Flower Development	1	1	1	1	2	3
Fruit Set on June 1st. %	92	91	77	67	92	25
June 24th. %	52	35	44	44	54	19
August 19th. %	46	35	35	33	44	17
Seeds Developed. %	33	21	14	13	30	11

The results of this experiment should be interpreted with some caution owing to the possible effect of external environmental factors, among which may be included soil conditions, climatic conditions and the attacks of insect and other pests. A gradual increase in percentage set for all treatments was noted between one end of the row of trees and the other, an effect which was almost certainly due to changes in the nutritional status of the trees.

Effect of the Use of Vaseline on Subsequent Fruit Set.

Comparison of treatments A and D shows that the use of vaseline (D) caused a distinct drop in percentage set and later in fruit development.

The phased treatments (D, E and F) reveal an optimum at Stage 2 with a sharp drop at Stage 3 similar to that obtained in Experiment 1. It is probable that at Stage 3, when the petals were beginning to fall, the stigmas were ceasing to be receptive.

Effect of the Artificial Bag Climate.

Inside the waterproof paper bag a humid atmosphere is rapidly produced and during sunny periods there is a considerable rise in temperature. In practice the developing fruitlets are subjected to this artificial climate for a period of two or three weeks until the bags are torn open.

Comparison of treatments B and C show that the trusses not enclosed in a bag (B) gave a higher initial set than those enclosed, but the number of fruits which ultimately developed was the same in both treatments.

The fruits exposed to the normal atmosphere remained noticeably smaller and more highly coloured than those enclosed in the bags. Large colonies of Rosy Apple Aphis (*Anuraphis roseus*) developed rapidly in some bags.

Direct Comparison of the Two Techniques.

The orthodox technique and the new technique are represented by treatments A and B respectively. Initially no difference was apparent between the treatments, but the post "June drop" and final counts show a higher percentage set for the orthodox technique (A).

No noticeable effect was produced by the omission of emasculation (C and D).

Discussion.

The use of vaseline as a protectant for the stigmas of artificially pollinated flowers appears from experiment and observation to be satisfactory. Vaseline applied in the field experiment remained intact for several weeks.

It is difficult to say if the vaseline itself has a directly harmful effect on pollen. By its use a reduction in germination percentage was observed "in vitro," but if, as normally occurs in practice, an excess of pollen is applied to the stigmas, this reduction might not seriously affect subsequent fertilisation.

Considering the results of Experiment 2 first; the reduction in fruit set for treatment D has already been noted, but under certain conditions in this experiment the percentage set obtained with the use of vaseline was as high as that obtained without its use. The results from treatment E where vaseline was applied at Stage 2 are almost identical with those obtained from treatment A where pollination was carried out at Stage 1 without the subsequent application of vaseline. Unfortunately at Stage 2 the flowers were open and thus this stage is too late for controlled pollination. Considering the timed treatments together, it is reasonable to suppose that at Stage 2 the maximum number of stigmas were receptive and consequently at Stage 1 a number were not fully receptive. Pollen sown on the non-receptive stigmas would have experienced a delay in germination. If during this period the natural reduction in viability of the pollen was greatly increased by the hermetic sealing by vaseline, then this might lead to a reduced set after early pollination. The good result obtained from treatment A, in which vaseline was not applied, indicates that any natural reduction in viability which occurred in the delay period did not adversely affect subsequent fruit set.

These deductions are not fully borne out by the results obtained in Experiment 1. In this experiment no set greater than 60% was obtained for any treatment. It is possible therefore that the severe limitations placed on the fertility of the tree by the conditions of pot culture may have had an over-riding effect on the fruit set obtained. The relatively good set obtained with the use of vaseline at Stage 1 in this experiment could be explained by assuming that the stigmas of Lord Lambourne became receptive at an earlier stage in the development of the flower than did those of Sweet Alford in the second experiment.

Because natural pollination can take place after the flowers have opened, controlled pollination cannot be delayed beyond the "balloon" stage of flower development and the fact that the use of vaseline at this stage may result in a reduced set must be borne in mind.

The main advantages to be obtained by not covering the trusses with a bag have already been mentioned. In the field experiment the open trusses gave the highest initial set, but there was a subsequent rapid drop in the number of fruits remaining on the trees so that the final counts for the two relevant treatments were the same. It is difficult to suggest an explanation for this. As vaseline was applied to the flowers in both treatments no after effect from such application can be assumed.

No detailed comparison of the labour involved in the two techniques was made. If the vaseline cream is contained in an open specimen tube, this can be conveniently carried with tubes of pollen in a satchel slung over the shoulder. The process of covering the stigmas is quick even when, as in the case of apples and pears, each flower possesses five stigmas. Although there appears to be a risk of a reduced set by using vaseline, the advantages of this method are such that it is definitely worthy of further trial.

Summary.

1. A description is given of a simplified method for controlled pollination of fruit trees. By protecting the flower stigmas with a smear of vaseline the use of a paper or muslin bag is avoided.

2. Experiments carried out with apple trees grown in pots and with established bush trees are described.

3. The application of vaseline appears to reduce pollen viability and in certain circumstances fruit set is also reduced.

4. The advantages of not subjecting flowers to an artificial bag climate are discussed.

5. Results obtained showed some variation, but were sufficiently promising to warrant further trial of this new method.

Acknowledgments.

Thanks are due to Dr. L. C. Luckwill for his helpful advice and to Miss Helen Ferres for assistance in planning the experiments.

REFERENCE.

- (1) SCHANDERL, H. (1949). Eine neue Methodik der Bestäubung von Obstblüten ohne Verwendung von Isoliertüten oder Musselinbeuteln. (A new method of pollinating fruit trees without isolating the flower by paper or muslin bags). *Züchter.*, **19**, 191-2.

A COMPARISON OF PRUNING TREATMENTS IN RELATION TO THE SHAPE AND YIELD OF APPLE TREES.

By R. M. JARRETT.

INTRODUCTION.

The earlier data on this experiment have been published in detail by Swarbrick and Berry in the Annual Report of 1937 (1), and further notes on crop yield by Swarbrick in the Annual Report of 1939 (2). These reports showed, that while any kind of winter pruning had reduced tree size, blossom production and crop, these reductions had been compensated for by better fruit quality and easier orchard management. Winter plus summer pruning had given some increase in fruit colour and size, but was accompanied by a further reduction in tree size and yield, and also had consumed much time. In the first of these reports (1) it was shown that with most varieties the open centre trees had produced heavier crops than the modified leader trees during this first nine-year period, but in the latter report (2), modified leader trees had given the bigger crops in the 1939 season.

This report will be mainly confined to crop data and observations comparing modified leaders with open centre trees.

Planting and Tree History.

The varieties used are Allington Pippin, Cox's Orange Pippin, Edward VII, Emneth Early (Early Victoria), and Worcester Pearmain, and were planted as one-year-old whips on Malling II stock in the winter of 1926. It should be noted that the present experiment commenced the third year after planting (1929) and that initially all the trees were treated as open centres, being pruned hard for two years.

Originally the trees were planted at 15 feet apart each way on the square. In 1937 Swarbrick (1) pointed out that 18 feet would have been a better spacing and by 1945 it had become necessary to remove alternate trees in both directions, thereby increasing the distance of each tree from its neighbours to 21 feet and reducing the number of trees on the plot by half. All varieties, except Worcester, are now using the increased area to the full, and gang mowing is just possible when the trees are carrying a full crop.

Treatments and Pruning.

The trees are in five categories, namely :

- | | | | | | |
|---------------------|----|----|----|----|---------------------------|
| I. Regulated | .. | .. | .. | .. | winter pruned. |
| II. Open Centre | .. | .. | .. | .. | winter pruned. |
| III. Open Centre | .. | .. | .. | .. | summer and winter pruned. |
| IV. Modified Leader | .. | .. | .. | .. | winter pruned. |
| V. Modified Leader | .. | .. | .. | .. | summer and winter pruned. |

(Trees receiving both summer and winter pruning will be referred to in the text as "summer pruned" trees).

Pruning continued as detailed in the 1937 Report (1) until 1945. It was then considered that, as tree thinning had reduced the number of trees by half and records had lapsed through the latter war years, the experiment had

yielded as much useful information as was likely to be obtained, and pruning method was altered considerably.

(a) *Regulated Trees*. Up to 1945 a minimum of cutting was practised in this group; pruning being confined to removal of crossing branches and diseased wood. In the winter of 1945-46 drastic branch thinning and much shortening of extended spurs was carried out, bringing the trees back to an open centre form, and since then these trees have been treated as such.

(b) *Open Centre Trees*. Up to 1945 these had been pruned on the strictly orthodox lines for shape, leader tipping and spurring of laterals in winter being practised. From 1945 leader tipping and spurring were discontinued and an attempt was made to change over to "Renewal Pruning," many laterals on Cox and Allington being left full length to be shortened the following year when they had spurred-up. At the same time older spurs were gradually reduced and replacement branches were encouraged.

(c) *Modified Leader Trees*. These trees were built up according to the principles laid down by Swarbrick (3) and consist of a central stem around which branches radiate at suitable positions along its length and as near the horizontal as possible. The tree was finally opened out at about 10 feet. Detailed pruning was similar to that practised on the open centre trees, i.e., the tipping of leaders and spurring of laterals. Trees of this shape had suffered considerably prior to tree thinning in 1945, due to lack of space for the lower branches, and from about 1942 these had been cut back, and up, to permit of cultivations. From 1945 renewal pruning was practised as for open centre trees, no attempt being made to alter the shape. It was, however, found necessary to reduce the number of "Y" forks in the lower branches to give ladder access to the tops of the trees, a point which had not been foreseen in the early formation of the modified leader tree.

Summer Pruning. Trees of both modified leader and open centre shapes were summer pruned annually until 1942. Pruning consisted of the cutting back of all the current year's growth to about five leaves, except the leaders which were left unpruned. This was usually carried out about the first week in August. During the winter the leaders were tipped and any secondary growths arising from the summer pruned shoots were cut to their basal buds. Apart from this summer pruning, treatment was the same as for modified leaders and open centres in the winter pruned group of trees, and since summer pruning ceased treatment has been identical for each tree shape. It is therefore only a delayed summer pruning effect which is apparent after 1942.

Cultivation and Manuring.

Clean cultivation was maintained from the date of planting until 1938 when the plot was grassed down and cut two or three times a year with a motor scythe. This cover was ploughed in following tree thinning in the Autumn of 1945 and re-sown in May, 1946 with a mixture of perennial rye grass (S.23), white clover (S.100), and red clover (S.123) at the rate of 12, 2, and 6 pounds per acre respectively, producing a dense sward which has since been gang-mown.

Manuring prior to grassing down was detailed in the 1937 Report (1). Manuring subsequently has been designed to meet changes in cultivation and wartime shortages of fertilisers. In 1938 and 1939 applications were of sulphate of ammonia, superphosphate and sulphate of potash at 3, 2 and 1 cwt. per acre respectively. During the war period (1940-45) nitrogen was applied as nitrochalk and did not fall below 2 cwt. per acre, rising to as much

as 5 cwt. on two occasions to encourage better shoot growth. Superphosphate was omitted twice and muriate of potash, which was used in place of sulphate, was omitted in four seasons, total applications, over the six years, being nitrochalk 20 cwt., superphosphate 11 cwt. and muriate of potash 3 cwt. per acre. Since the war, and with gang mowing of the sward, the following fertiliser applications have been sufficient to give good shoot growth and a rapid increase in tree size : 2-3 cwt. of nitro-chalk, 3 cwt. of superphosphate and 2 cwt. of muriate of potash per acre annually.

Records.

These were drastically curtailed by conditions resulting from the war. After 1940 only crop weights were kept, these also ceasing with the 1942 crop to be recommenced in 1946 for a further three years. It then became apparent that there was now insufficient replication to give significant differences and all records ceased in 1948. As the records of girth, tree measurements and blossom counts, etc., were fully dealt with in the 1937 Report (1) up to that time, and later records only continued for a further two or three years and give no extra information they are not included here.

Crop Weights.

Cropping of the variety Cox has been adversely affected by a progressive increase in canker, which in the early years was confined to the main limbs, but in later years caused much annual loss of young wood. This was not confined to any one treatment and has affected total yields, particularly in the 1946-48 period, which are very low. The variety Edward, under conditions at Long Ashton, is rather slow in coming into full bearing, and makes large trees which crop heavily following tree thinning, when about 20 years old. This has been observed elsewhere on the station and would account for the very high yields in the 1946-48 period.

To overcome, to some extent, the limitations imposed by systematical planting (described by Swarbrick (1)), and make Analysis of Variance more valid, each variety has been treated separately in this report. This has resulted in some differences shown in the earlier report (1) not now being significant, and no inter-varietal comparisons are made. Also, it has been necessary to compute statistically the yields of a few trees which have died from various causes during the course of the experiment.

For ease of comparison of this report with that of 1937, and to show approximately where the cropping order of treatments changes (this is shown better by the graph (Fig. 1), and is discussed later), the yields for the total period 1929 to 1942 have been divided into two periods. The first of these is from 1929 to 1937 or the period covered by the first report, and the second is 1938 to 1942, the final period under the original treatments. A later period, 1946 to 1948, is given, but only to show that the trend of the modified leader and open centre trees, established in the early periods has been continued in most cases ; for, as already stated, records had lapsed between 1943 and 1945, treatments had altered (mainly treatment I), and these later yields show no significant difference within any variety except Emneth, due to lack of replication caused by tree thinning.

The weights of marketable fruit for each tree were totalled for the successive years in each period and the mean per tree calculated for the period ; these are expressed in kilograms in Table I and are convertible to bushels per acre by multiplying by 10 (1929-42) or 5 (1946-48). Analysis of

TABLE I.
EFFECT OF PRUNING AND SHAPING ON YIELD OF FRUIT.
WEIGHT IN KILOGRAMS PER TREE :- { PERIODS 1929 to 1942. KILOS/TREE $\times$ 10 = BUSHELS PER ACRE APPROX.
PERIOD 1946 to 1948. KILOS/TREE $\times$ 5 = BUSHELS PER ACRE APPROX.

Treatment	I. Regu- lated	II. Open Centre	III. S. Pruned O.C.	IV. Mod. Leader	V. S. Pruned M.L.	Mean	Sig. Diff.		All Trees		All Trees		Sig. Diff.		Differences * = at 5% P. ** = at 1% P.
							1% P.	5% P.	O.C.	M.L.	W. P. only	W. & S. Pruned	1% P.	5% P.	
<i>Allington</i>	1929-37	252.7 (1)	186.5 (2)	164.3 (5)	171.1 (3)	165.2 (4)	188.0	No Sig. Diff.	175.4	168.1	178.8	164.7	—	13.5	* W.P. > W. + S.P.
	1938-42	305.5 (1)	203.0 (4)	176.1 (5)	235.5 (2)	230.3 (3)	230.1	44.0	189.5	232.9	219.3	203.2	31.3	23.2	** I > II, III, IV & V. V & IV > III. ** M.L. > O.C.
	1929-42	558.2 (1)	389.5 (4)	340.4 (5)	406.6 (2)	395.5 (3)	418.1	59.6	364.9	401.0	398.1	367.9	—	32.2	** I > II, III, IV & V. IV > III. * II > III. M.L. > O.C.
	1946-48	113.7 (2)	113.5 (3)	102.5 (5)	126.7 (1)	102.6 (4)	111.8	No Sig. Diff.	108.0	114.7	120.1	102.5	No Sig. Diff.		
<i>Cox</i>	1929-37	155.8 (1)	141.6 (2)	102.8 (4)	119.9 (3)	89.6 (5)	122.0	43.6	122.2	104.8	130.8	96.2	27.4	21.2	** I > III & V. II > V. W.P. > W. + S.P. * I > IV. II > III & V.
	1938-42	154.9 (2)	122.0 (4)	109.0 (5)	187.5 (1)	142.2 (3)	143.1	31.8	115.5	164.8	154.7	125.6	24.0	17.8	** IV > I, II, III & V. I > II & III. V > III.
	1929-42	310.7 (1)	263.6 (3)	211.8 (5)	307.4 (2)	231.8 (4)	265.1	68.5	237.7	269.6	285.5	221.8	40.4	29.9	** M.L. > O.C. W.P. > W. + S.P. ** I & IV > III & V. W.P. > W. + S.P.
	1946-48	41.3 (5)	45.2 (4)	59.2 (1)	58.8 (2)	50.3 (3)	51.0	No Sig. Diff.	52.2	54.6	52.0	54.9	No Sig. Diff.		* II > III. M.L. > O.C.

<i>Edward</i> 1929-37	68.0 (2)	73.5 (1)	52.4 (5)	66.5 (3)	54.4 (4)	62.9	—	13.7	63.0	60.4	70.0	53.4	13.5	10.0	** W.P. > W. + S.P.
1938-42	199.9 (5)	246.1 (2)	219.2 (4)	254.6 (1)	221.4 (3)	228.3	No Sig. Diff.	232.6	238.0	250.4	220.3	No Sig. Diff.			
1929-42	267.9 (5)	319.6 (2)	271.6 (4)	321.1 (1)	275.8 (3)	291.2	No Sig. Diff.	295.6	298.5	320.4	273.7	—	38.8		* W.P. > W. + S.P.
1946-48	462.9 (1)	461.0 (2)	409.3 (5)	409.6 (4)	457.6 (3)	440.1	No Sig. Diff.	435.1	433.6	435.3	433.4	No Sig. Diff.			
<i>Emmethyl</i> 1929-37	178.4 (1)	152.7 (2)	138.7 (4)	148.9 (3)	128.7 (5)	149.5	28.8	21.6	145.7	138.8	150.8	133.7	—	15.5	** I > IV, III & V. * I > II > V. W.P. > W. + S.P.
1938-42	302.9 (1)	220.0 (3)	187.7 (5)	238.6 (2)	197.2 (4)	229.3	37.4	28.0	203.9	217.9	229.3	192.5	29.3	21.7	** I > II, III, IV & V. IV > III & V. ** W.P. > W. + S.P. * II > HL.
1929-42	481.3 (1)	372.7 (3)	326.4 (4)	387.5 (2)	325.9 (5)	378.8	52.0	38.9	349.6	356.7	380.1	326.2	38.0	28.2	** I > II, III, IV & V. IV > III & V. ** W.P. > W. + S.P. * II > III & V.
1946-48	212.7 (4)	278.5 (1)	233.5 (3)	234.5 (2)	201.0 (5)	232.1	—	42.8	256.0	217.7	256.5	217.3	—	32.6	* II > I, III, IV & V. O.C. > M.L. * W.P. > W. + S.P.
<i>Worcester</i> 1929-37	105.7 (1)	85.3 (2)	82.3 (3)	73.1 (4)	62.3 (5)	81.7	18.3	13.6	83.8	67.7	79.2	72.3	11.7	8.7	** I > II, III, IV & V. II & III > V. ** O.C. > M.L.
1938-42	175.7 (1)	163.9 (3)	166.7 (2)	161.6 (4)	151.6 (5)	163.9	No Sig. Diff.	165.3	156.6	162.8	159.2	No Sig. Diff.			
1929-42	281.4 (1)	249.2 (2)	249.0 (3)	234.7 (4)	213.9 (5)	245.6	—	36.9	249.1	224.3	242.0	231.5	—	22.8	* I > IV & V. O.C. > M.L.
1946-48	176.8 (3)	196.0 (1)	192.7 (2)	175.2 (4)	166.5 (5)	181.4	No Sig. Diff.	194.3	176.8	185.6	179.6	No Sig. Diff.			

variance was carried out for each period and divided into two sections, the first being an analysis of all treatments, and the second of treatments II, III, IV, and V only; i.e., leaving out the regulated treatment. From this latter analysis, effects attributable to summer pruning can more readily and accurately be assessed; as also, can modified leaders be contrasted with open centre trees. In no case was the shape $\times$ pruning interaction significant.

Allington. Regulated trees have given the highest yields in both the first and second periods, being highly significant ($P=0.01$) in both the second and total periods, but not in the first. Comparing open centre trees with modified leaders, it will be seen that although the open centre trees had the lead in the first period, by 1942 modified leader trees had given considerably higher yields and these differences are highly significant ($P=0.01$) in the second period, and significant ($P=0.05$) over the total period. This lead was maintained in the 1946-48 period. Summer pruning reduced the crop in all periods, but only significantly so in the first period, other periods being only slightly above the significant point.

Cox. Regulated trees have given significantly higher yields in the first period, but take second place to modified leader winter pruned trees in the second period, and over the total period are very little different, both treatments giving higher yields than the open centre and modified leader summer pruned trees. All these differences are significant at $P=0.01$. Comparing open centre with modified leader trees, the latter have given better yields in the second and over the whole period, being significant at the 0.01 and the 0.05 value of P respectively. Open centre trees were higher in the first period, but not significantly so. Summer pruning has significantly reduced the crop in each period ($P=0.01$).

Edward. With this variety the only significant effect is that of summer pruning, which reduced the crop in all periods, being significant at the 0.01 value of P in the first and at the 0.05 value over the whole period. This variety shows marked tree-to-tree variation and there is no doubt that this prevents other differences from reaching significance. It is interesting, however, to notice that there is little difference attributable to shape in any period, and that the regulated trees take the lowest position in the second and total period, rising to first position in the 1946-48 period. This followed a severe pruning in 1945 when these trees were changed over from what was practically "No Pruning," to something approaching a normal open centre shape.

Worcester. Regulated trees have given the highest yields from the beginning until 1942, these being significant at $P=0.01$ in the first period and at $P=0.05$ over the total period. No treatments show any significant effect in the second period. Comparing open centre trees with modified leaders, the former have given higher yields throughout all periods, being significant at $P=0.01$ in the first and at $P=0.05$ over the whole period. Summer pruning just failed to reduce yields sufficiently, in all periods, to the significant point.

Emmeth. Regulated trees in each period up to 1942 have given yields significantly exceeding all other treatments at $P=0.01$. Summer pruning has reduced yields below those of winter pruned trees over the same period at $P=0.01$ and in the 1946-48 period at $P=0.05$. It is only in this latter period that tree shape shows any significant difference, and here the open centres crop more heavily than modified leader trees at a significance of $P=0.05$.

Over the second period, 1938-42, two pickings of the crop of this variety were carried out and a record made at each picking date. The first pick

TABLE II.
VARIETY : EMNETH EARLY (EARLY VICTORIA).
EFFECT OF TREATMENTS ON FIRST AND SECOND PICK (PERIOD 1938-42).
WEIGHT IN KILOGRAMS PER TREE.

Treatment	I. Regulated	II. Open Centre	III. S.Pruned O.C.	IV. Mod. Leader	V. S.Pruned M.L.	Mean	Sig. Diff.		All Trees		All Trees Winter P. only	Sig. Diff.		Differences { * = at 5% P. ** = at 1% P.	
							1% P.	5% P.	O.C.	M.L.		1% P.	5% P.		
Pick. 1st	88.3 (5)	122.6 (2)	98.4 (4)	144.8 (1)	109.1 (3)	112.6	26.7	20.1	110.5	126.9	133.7	103.8	23.0	17.2	** IV > V, III & I. II > I. W.P. > W. + S.P.
															* IV > II > III & I. V > I.
2nd	214.6	97.4	89.3	93.8	88.1	116.7	26.7	20.1	93.4	91.0	95.6	88.7	23.0	17.2	** I > II, III, IV & V.
Total	302.9	220.0	187.7	238.6	197.2	229.3	37.4	28.0	203.9	217.9	229.3	192.5	29.3	21.7	(As in Table I).

usually took place during the last few days in July each year and consisted only of fruits that were of first quality market size; smaller fruits being left on the tree to size up for the second pick, which was taken a fortnight to three weeks later when the whole of the remaining crop was gathered. These figures are presented in a similar manner to Table I in Table II and a Split Plot Analysis of Variance has been applied. Referring to Table II, it will be noticed that although the regulated trees gave the highest total yield, the bulk of this is in the second pick, which is significantly higher than any other treatment at a greater probability level than $P=0.01$, whilst the first pick is lowest of any, significantly so except for the open centre summer pruned treatment. This gives a fair indication of the fruit size to be expected from regulated trees with all varieties. With other treatments, the weight of the first pick exceeds the second in every case, all differences being significant with the exception of the open centre summer pruned trees. In the second pick, apart from the regulated trees, there is very little difference between treatments and it may be said that any difference in the total yield is attributable to the first pick. Modified leader winter pruned trees yield significantly more ($P=0.01$) than any other treatment with the exception of the open centre winter pruned trees, which are also exceeded at $P=0.05$. Summer pruning has again significantly reduced the yield in the first pick, being significant at $P=0.01$.

The annual and total yield for the 14 years 1929 to 1942 has been calculated on a tree basis for each treatment and variety and is expressed on the graph, Fig. 1. It has been necessary to group some of the earlier annual yields, which were small, and these are shown as the yield of a single year, there being no change in the pattern which normally indicates annual divisions in the scale. Similarly, 1935, a complete frost year, is not shown.

Generally, summer pruning has had the effect of depressing yield from early in the cropping life of the trees, with every variety and either tree shape. Conversely, regulated pruning has given the highest yields from an early age with all varieties except Edward. Differences due to shape begin to appear further up the scale, i.e., later, and the initial advantage is generally with the open centre trees; but whilst this position is maintained right through to 1942 in the case of Worcester, with Cox and Allington, modified leader trees take top place after 1939 and 1940 respectively, having in these years approximately equalled the open centres in accumulated total yield.

Comparing varieties, it will be seen that Allington has given the heaviest crop over the 14 years, followed fairly closely by Emneth (Early Victoria), then Edward, Cox and Worcester in that order. Expressing these as mean annual yields of all treatments for the 14 years:—

Allington	..	314	} = Bushels per acre per annum (1929–1942).
Emneth	..	284	
Edward	..	218	
Cox	..	199	
Worcester	..	184	

Biennial Bearing.

In an endeavour to find what effect, if any, the pruning treatments had had on biennial bearing the varieties Allington and Emneth were selected as being notably subject to this habit. The methods of estimation used are the indices "I" and "B" devised by Hoblyn, Grubb, Painter and Wates (4), with the modification of Wilcox (5), and described by Singh (6) as follows:—

PLATE I



Open Centre tree,
Var. Worcester Pearmain, Winter 1950-51.



Open Centre tree,
Var. Allington Pippin, Winter 1950-51.



Modified Leader tree,
Var. Worcester Pearmain, Winter 1950-51.



Modified Leader tree,
Var. Allington Pippin, Winter 1950-51.

APPLE TREE PRUNING AND SHAPING ANNUAL AND TOTAL YIELDS 1929-1942

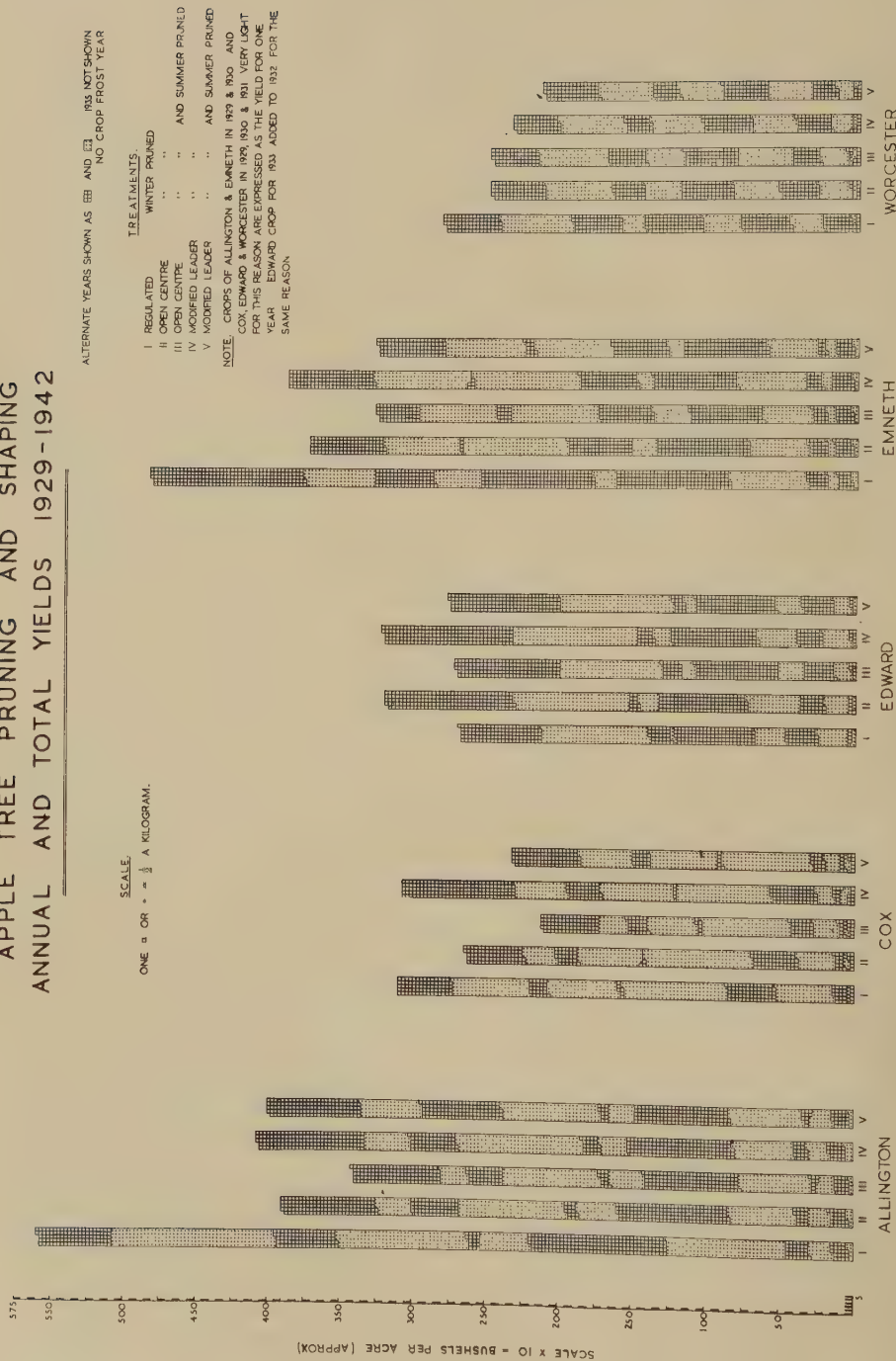


Fig. 1.

"To determine 'B' the yield for each year is considered in relation to that of the previous year. If the second yield exceeds the first, the difference is given a plus sign, and if the converse, a minus sign. Thus a series of signs for each consecutive pair of years is obtained. From these, 'B' is calculated by determining the percentage of consecutive pairs of unlike signs over the whole period. A value of 'B'=100 is obtained when the tree is completely biennial, i.e., when + is followed by —, and vice versa, throughout the period. On the other hand, when 'B'=0 the tree is regularly increasing (or regularly decreasing) in yield. The second factor 'I' was employed as a measure of the intensity of degree of crop fluctuation from year to year." This may, with Wilcox's modification, be expressed as :—

$$I = \frac{(\text{difference between successive yields})}{(\text{sum of successive yields})} \times 100$$

A value of "I" = 100 is obtained when the fluctuation is such, that one of the successive yields is zero. Conversely, when "I" = 0 it indicates no fluctuation in successive years.

Crops in the year 1935 being a complete failure due to frost, it would be expected that this would induce biennial bearing in varieties subject to the habit. Accordingly, the annual yields of Allington and Emneth were examined for the period 1936 to 1942 inclusive, and the indices "B" and "I" calculated for each tree. These are summarised in Table III, which gives the mean value for each treatment.

TABLE III.
BIENNIAL BEARING INDICES "B" AND "I"; PERIOD 1936 TO 1942.
SUMMARY OF MEAN VALUES.

Treatment	Allington		Emneth	
	"B"	"I"	"B"	"I"
Regulated Winter Pruned	80	55	72	57
Open Centre Winter Pruned	62	54	73	75
Open Centre Winter and Summer Pruned	62	55	78	66
Modified Leader Winter Pruned	68	56	75	73
Modified Leader Winter and Summer Pruned	65	56	76	69

From this table it will be apparent that the variety Allington receiving "regulated" pruning gives a large "B" index indicating a marked biennial habit, but the "I" index is not very high, and thus fluctuations from year to year are not as great as would be expected where biennial bearing is in full swing. For other treatments, the lower "B" with similar "I" indices indicate similar amounts of fluctuation, but these are not of a true biennial nature. The variety Emneth would appear to be generally more biennial than Allington, but unlike the latter, there is no effect on habit attributable to "regulated" treatment, or any other; all treatments showing nearly similar "B" indices. However, there would appear to be some small differences in the intensity of the fluctuations, as indicated by "I." This shows a medium

value for the regulated trees and a fairly high value for either shape when winter pruned only. The addition of summer pruning appearing to reduce intensity slightly.

To sum up, Emneth appears to be rather more biennial than Allington. Tree shape has had no effect on either variety. On Emneth, summer pruning appears to have slightly reduced the fluctuations from year to year, some further evening-up being apparent on trees receiving regulated treatment. For Allington, the only treatment difference is that of "regulated," which appears to induce a more biennial habit than other treatments, these latter fluctuating as much, but not biennially.

Quality.

This was dealt with in some detail in the 1937 Report (1) and showed only small differences. In the 1939 Report (2) Swarbrick again graded all the fruit from each treatment and reports that "... there was practically no difference in the percentage of fruit falling into each size and colour grade as a result of pruning treatments." Since then no further attempt has been made to measure the quality of fruit from these trees, and from visual observations there appears to be little difference between fruit from modified leader trees and that from open centre trees. Regulated trees do, however, give a predominance of small fruits, as indicated by the comparison of the two picks of Emneth in this report; and it would appear that spray control measures are not so effective on these trees, resulting in more scab and other damage to the fruit.

Discussion.

Ignoring the small amount of pruning applied to the regulated trees, any form of pruning has generally reduced yields, but while with winter pruning this reduction is offset by an improvement in quality and easier orchard management, summer pruning has further reduced yields with no very apparent improvement in quality. Summer pruning is therefore not to be recommended for bush plantations where the aim is to produce large trees with heavy crops at an early age, although it may be needed on cordons and dwarf pyramids for restricting vigour.

Comparing the two shapes of tree, modified leader trees have given ultimately higher yields with Allington and Cox, but Worcesters have cropped better as open centre trees. The other two varieties gave little difference during the period of the original treatments, but Emneth shows an increased crop from open centre trees in a later period when renewal pruning was practised. It would seem that the compact habit of Worcester does not lend itself to the horizontal branch system of the modified leader tree; whereas Cox and Allington, being of a more spreading habit do so, and might possibly have attained this ascendancy earlier in their life but for the fact, already stated, that the open centre trees had two years advantage over the modified leaders, and drastic changes were necessary to develop as modified leaders, trees which had been started as open centres.

Comparing the physical advantages and disadvantages of both shapes, it was claimed in the first report (1) that modified leader trees had an advantage of accessibility for picking etc. over the open centre, but this disappeared with the ageing of the tree. As the trees grew, the lower branches became more rigid and it became difficult to place steps near the axis of the tree. This could have only been overcome by building fewer main branches in the lower

tiers, and/or not allowing these branches to fork. Also the height could with advantage have been reduced by opening the centres before the trees reached 10 feet, resulting in an overall height of about 15 feet. Many of the open centre trees have also attained this height, but do not present the same difficulties in picking, for here height is accompanied by an outward slant which is increased by the weight of fruit when the tree is in crop. From the point of view of cultivations, the spreading rigid horizontal branches of the modified leader tree can be a nuisance, for while implements can be "off-set" to go beneath the trees, the tractor must have a reasonable avenue down which to travel. With the open centre trees this difficulty is only encountered during the latter weeks of a season of heavy crops; but at this time modified leader trees are rather better, for the rigid horizontal branches arising from a wide angle crotch sag very little and will not break; a point claimed in their favour by Swarbrick (1) and (3).

It will be seen that both shapes have advantages and if these could be embodied in one type of tree without the attendant disadvantages some progress would have been made. The more recently introduced Delayed Open Centre tree seems a possible solution to this problem, for it combines the crotch strength and the light early pruning of the modified leader tree with the accessibility of the open centre, branch slope being somewhere between the two treatments. It has not the disadvantages of the modified leader tree here described, for the centre is generally opened quite early in the tree's life and lower branches are considerably fewer.

Summary.

1. An assessment of the relative value of certain pruning treatments on five varieties of apple is continued from the 1937 Report (1). These treatments were designed to compare the performance of trees of modified leader and open centre shapes, and to compare the effects of summer-plus-winter pruning with winter pruning only on these tree shapes, and also with regulated trees.

2. Crop weights for different pruning treatments are compared statistically for each variety separately. Any pruning reduces yield, but a gain in quality is achieved by normal winter pruning. This, plus summer pruning, has further reduced the crop with no further increase in quality. Of the two shapes, Allington and Cox produce ultimately bigger crops when treated as modified leader trees, but the reverse is true for Worcester which cropped better as an open centre tree.

3. Varieties are compared non-statistically and give the following order of cropping, from highest to lowest:—Allington, Emneth (Early Victoria), Edward, Cox and Worcester.

4. An attempt is made to find where in the life of these trees treatment differences first became apparent in crop yield. This shows that differences due to regulated and summer pruning became evident in the early years, but differences due to shape did not appear until much later.

5. On the varieties Emneth and Allington, tree shape does not affect liability to biennial bearing. On Emneth, summer pruning slightly reduces yearly fluctuations: on Allington, "regulated" pruning induces a higher degree of biennial bearing than the other pruning treatments.

Acknowledgments.

The writer is much indebted to many of his colleagues for their assistance, particularly to the following: Mr. E. W. Hobbis (at whose instigation the

writing of this report was undertaken); his constant help and ideas have been freely given and used; to Dr. L. C. Luckwill for reading and suggesting additions to the original script; to Miss H. Ferres for her guidance in the statistical methods used in the interpretation of the data presented, and also, to Mr. E. Catlow who has assisted throughout the investigation.

LITERATURE CITED.

- (1) SWARBRICK, T. and BERRY, W. E. Comparison of pruning treatments in relation to apple tree shape: Progress Report. *Ann. Rept. Long Ashton Res. Sta.*, 31-56, 1937.
- (2) SWARBRICK, T. The effect of pruning for tree shape upon the yield of apples. *Ann. Rept. Long Ashton Res. Sta.*, 20-23, 1939.
- (3) SWARBRICK, T. The aims, advantages and method of building a modified leader tree. *Sci. Hort.*, **6**, 299, 1938.
- (4) HOBLYN, T. N., GRUBB, N. H., PAINTER, A. C., and WATES, B. L. Studies in biennial bearing. *J. Pomol.*, **14**, 39-76, 1936.
- (5) WILCOX, J. C. Some factors affecting apple yields in the Okanagan Valley. *Sci. Agric.*, **25**, 189-213, 1944.
- (6) SINGH, L. B. Studies in biennial bearing—II. A review of the literature. *J. Pomol.*, **24**, 45-65, 1948.

A MANURIAL EXPERIMENT ON BLACKCURRANTS. PROGRESS REPORT, III.

By C. BOULD and E. CATLOW.

The object and design of this experiment were described in Progress Report I. (1). A second Progress Report (2) dealt with information on growth, yield and leaf analysis up to 1947. The present report summarises the results over the seasons 1946-50. By 1950 the bushes had become so large, due to the light system of pruning adopted, that cultural and picking operations were difficult. In order to extend the economic life of the plantation, drastic pruning was essential. It was realised that this severe pruning might mask the response to further manurial treatments in future years. The experiment has therefore reached a stage when it is convenient to summarise the results to date.

Experimental.

Manures.

From planting till 1948 the bulky organic manures were ploughed in during January or February by tractor. Since then they have been worked in by harrowing, usually during March. The other fertilizers and manures were applied in March.

Sprays.

The bushes received an annual spray of 0.1% D.N.O.C. in 5% oil, and 2% lime sulphur in 1945, '48 and '50. A copper spray (Bordeaux, Coppesan or colloidal copper) was given each year after fruit picking in an attempt to control leaf spot.

Pruning.

Light pruning was practised in order to minimise interference with manurial effects. Growth was good throughout and by 1948 bushes receiving high nitrogen treatments were occupying fully the 8' x 4' planting distance. At the end of the 1950 season, it was decided to prune hard and low, more especially with the variety Cotswold Cross. The amount of wood removed in 1950 was approximately equal to the total amount for the previous four seasons.

Pests and Diseases.

The bushes generally have been remarkably free from pests.

Leaf spot has been the worst disease and was particularly severe in 1947, '49 and '50. Reversion was slight, 20 bushes of 504 in Cotswold Cross and 13 bushes of 620 in Mendip Cross becoming affected during the period.

Manures : Rates and Nitrogen Contents.

Tables 1a and b show the quantities of manure, together with amounts of total nitrogen, applied per acre each year. The organic manures were given on an equal organic matter basis, equivalent to 10 tons per acre (fresh weight) of F.Y.M. It will be noticed that the amounts of nitrogen varied

considerably between and within treatments from season to season. Furthermore, the bulky organics supplied more total nitrogen than nitro-chalk and meat and bone meal. The two latter treatments supplied equal amounts of nitrogen.

TABLE IA.

BLACKCURRANT MANURIAL EXPERIMENT PLOT 7A. FRESH WEIGHT OF MANURES APPLIED AND TOTAL NITROGEN CONTENTS IN TONS AND LBS. PER ACRE RESPECTIVELY. 1945-1950

VAR. COTSWOLD CROSS.

Year	1 F Y.M.		2 Sewage Sludge		3 Town Refuse Compost		4 Meat and bone Meal		5 Nitro-chalk	
	Fr. wt. T.p.a.	N lbs.p.a.	Fr. wt. T.p.a.	N lbs.p.a.	Fr. wt. T.p.a.	N lbs.p.a.	Fr. wt. T.p.a.	N lbs.p.a.	Fr. wt. T.p.a.	N lbs.p.a.
1945	10	—	12.9	—	12.6	—	0.51	104	0.3	104
1946	10	161	16.2	292	27.9	421	0.51	104	0.3	104
1947	10	106	9.0	190	13.1	198	0.58	104	0.3	104
1948	10	171	8.8	242	10.5	129	0.48	104	0.3	104
1949	10	89	5.9	117	10.7	73	0.50	104	0.3	104
1950	10	157	13.2	—	30.0	374	0.50	104	0.3	104

TABLE IB.

VAR. MENDIP CROSS.

Year	1 F.Y.M.		2 Sewage Sludge		3 Straw-sludge Compost		4 Straw-nitro- chalk compost		5 Nitro-chalk	
	Fr. wt. T.p.a.	N lbs.p.a.	Fr. wt. T.p.a.	N lbs.p.a.	Fr. wt. T.p.a.	N lbs.p.a.	Fr. wt. T.p.a.	N lbs.p.a.	Fr. wt. T.p.a.	N lbs.p.a.
1945	10	—	12.9	—	13.5	—	14.7	—	0.3	104
1946	10	161	16.2	292	12.3	204	17.6	165	0.3	104
1947	10	106	9.0	190	10.7	161	11.6	100	0.3	104
1948	10	171	8.8	242	10.3	204	14.8	115	0.3	104
1949	10	89	5.9	117	6.2	136	9.2	59	0.3	104
1950	10	157	13.2	—	13.0	206	15.0	67	0.3	104

N.B.—Organics applied on an equivalent organic matter basis equivalent to 10 tons p.a. fresh weight of F.Y.M.

Results.

The mean values for leaf nitrogen, crop yield and pruning weights for the seasons 1946-50, together with tests of significance, are given in Tables IIa and b.

TABLE IIA.

BLACKCURRANT MANURIAL EXPERIMENT, PLOT 7A. MEAN VALUES FOR LEAF NITROGEN, CROP YIELD AND PRUNING WEIGHTS FOR THE SEASONS, 1946-50.

VAR. COTSWOLD CROSS.

Mean Value	1 F.Y.M.	2 Sewage Sludge	3 Town Refuse Compost	4 Meat & Bone Meal	5 N.P.	6 Control	S.E. Mean	Sig. dif. P = .01
N as % Leaf- Dry Wt. . .	2.57	2.83	2.67	2.83	2.88	2.44	0.019	0.072
Crop yield- Tons p.a. . .	3.27	3.62	3.31	3.66	3.72	2.67	0.098	0.365
Pruning weights- Tons p.a. . .	2.28	2.89	2.46	3.00	3.18	1.74	0.082	0.307

For P = .01 { Nitrogen— Treatments 5, 4, 2 > 3 > 1 > 6.
 Crop yield— „ 5 > 1, 3, 6; 4 > 1, 6; 2, 3, 1 > 6.
 Pruning Weights— „ 5, 4, 2 > 3, 1 > 6.

TABLE IIB.

VAR. MENDIP CROSS.

Mean Value	1 F.Y.M.	2 Sewage Sludge	3 Town Refuse Compost	4 Straw Nitro- Chalk Compost	5 N.P.	6 Control	S.E. Mean	Sig. dif. P = .01
N as % Leaf- Dry Wt. . .	2.47	2.69	2.56	2.55	2.80	2.41	0.015	0.054
Crop Yield- Tons p.a. . .	3.26	3.30	3.05	3.14	3.58	2.63	0.072	0.267
Pruning Weights- Tons p.a. . .	2.13	2.73	2.05	2.15	2.62	1.58	0.087	0.224

For P = .01 { Nitrogen— Treatments 5 > 2 > 3, 4 > 1 > 6.
 Crop yield— „ 5 > 2, 1, 4, 3 > 6.
 Pruning Weights— „ 2, 5 > 4, 1, 3 > 6.

Leaf samples were taken prior to fruit picking. Yields of fruit and pruning weights are based on the means from plots containing 21 experimental bushes (including replants).

The effect of treatments on leaf nitrogen and crop yield is illustrated in Figs. 1 and 2. It will be noticed that the leaf nitrogen status was maintained at about the same level for the first two seasons, after which it began to fall.

There was no significant difference between the availability of nitrogen in nitro-chalk and meat and bone meal as measured by the effect on leaf nitrogen. The availability of nitrogen in the bulky organics was less than in the concentrated manures.

Previous results (2) indicated that there might be a positive correlation between the leaf nitrogen content prior to fruit picking and the yield of fruit in the following year. It was also suggested that the optimum leaf nitrogen

EFFECT OF TREATMENTS ON LEAF NITROGEN STATUS.

VAR. MENDIP CROSS.

VAR. COTSWOLD CROSS.

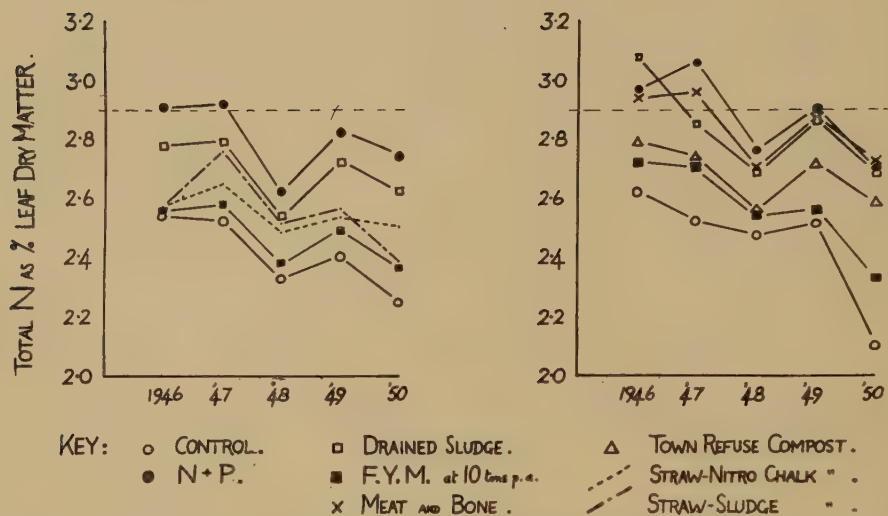


Fig. 1. Showing the effect of manurial treatments on the nitrogen status of blackcurrant leaves. Leaves sampled prior to fruit picking. The horizontal broken line indicates a tentative "optimum" value.

EFFECT OF TREATMENTS ON CROP YIELD 1946-50.

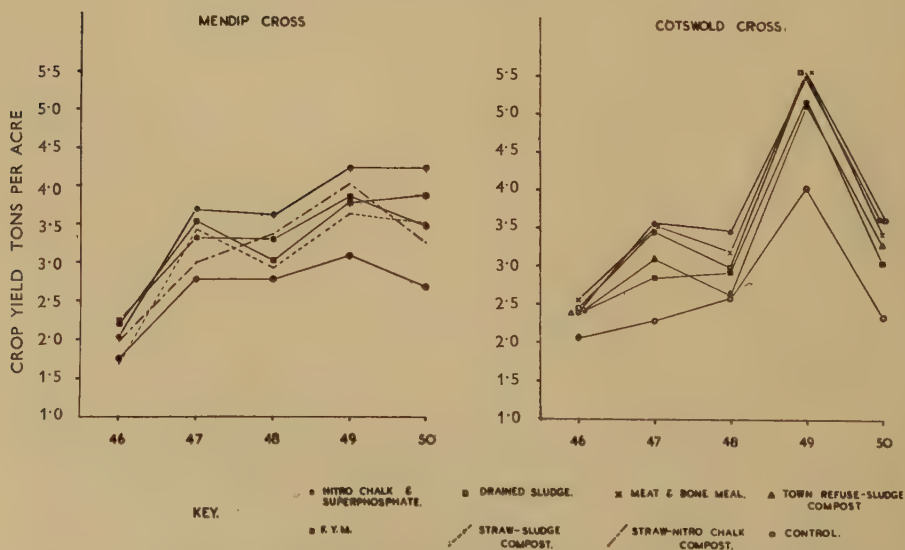


Fig. 2. Showing the effect of manurial treatments on the yield of fruit over a number of years from two varieties of blackcurrant.

content at this stage for the two varieties, providing no other nutrient was limiting growth, was in the region of 2.9% of the dry matter. The horizontal dotted line in Fig. 1 indicates this tentative "optimum" value. With the variety Mendip Cross none of the treatments maintained this "optimum" level after 1947, whereas with the variety Cotswold Cross, three treatments, nitro-chalk, meat and bone meal and sewage sludge (with the exception of low values in 1948) maintained this level till 1949. It would appear from these results that the variety Mendip Cross has a higher nitrogen requirement than Cotswold Cross.

Correlations between leaf nitrogen and yield of fruit over a number of years have not yet been worked out, but comparison between Figs. 1 and 2 indicate that the relative orders of yield and leaf nitrogen are similar. With a perennial crop such as blackcurrant the size of bush and yield of fruit increase each year until a maximum is reached, whereas if the treatments are fixed the same amount of nitrogen has to support an increasing size of plant. With limited applications of nitrogen, the leaf nitrogen content must thus fall. This is brought out in Fig. 1.

The effect of manurial treatments on the quality of the crop has been studied by Pollard *et al.* (3). They found that an increase in the uptake of nitrogen by the bush was associated with a lowering of the ascorbic acid content of the fruit, but, because of the increased yield of fruit from the high nitrogen bushes, the total ascorbic acid per acre was greater.

In commercial practice it might be better to give smaller dressings of nitrogen when the bushes are young and to increase progressively the applications annually so as to maintain an optimum leaf nitrogen status throughout the life of the bush. The "optimum" value will no doubt vary with the variety and general nutrient status of the plant. Further work is in progress on this problem.

Summary.

1. The management of a manurial experiment on two varieties of blackcurrant, Cotswold Cross and Mendip Cross, is outlined.
2. Results are given for the effect of treatments on leaf nitrogen, crop yield and pruning weights for the seasons, 1946-50.
3. The results show that the availability of nitrogen in nitro-chalk and meat and bone meal, when used on an equivalent nitrogen basis, is similar. The availability of nitrogen in F.Y.M. and composts is much lower than in meat and bone meal.
4. The order of yields follows closely the leaf nitrogen status.

Acknowledgments.

The sewage sludge was supplied by the Bath City Council (Engineer's Dept.), and the town refuse compost by the London County Council. The co-operation of these Authorities is gratefully acknowledged and best thanks are due to Mr. J. Owens, City Engineer and Mr. N. Priest, Sewage Works Manager, Bath, and to Mr. Warlow of the L.C.C.

Thanks are also due to Miss P. Scadding for help with the analytical work, to Mr. Hobbs for advice on management, and to Mr. Jarrett, Mrs. Lewis and Miss Woodhouse for help with the management and recording.

REFERENCES.

- (1) BOULD, C. and CATLOW, E. (1947). A manurial experiment on blackcurrants. Progress Report I. *Long Ashton Res. Stn., Ann. Rept.*, 1946, 31-36.
- (2) BOULD, C. and CATLOW, E. (1948). Progress Report II. *Long Ashton Res. Stn. Ann. Rept.*, 1947, 52-58.
- (3) KIESER, M. E., POLLARD, A. and STONE, A. M. (1950). The effect of manurial treatments on the composition of blackcurrants and their products. *Long Ashton Res. Stn. Ann. Rept.*, 1949, 159-162.

A NOTE ON BORON IN RELATION TO BITTER PIT IN APPLES.

By C. BOULD and J. TOLHURST.

Some years ago Wallace and Jones (3) carried out an investigation to find whether any relationship existed between the boron status of apples and the incidence of Bitter Pit. They were unable to find any relationship between the two. Recently however, Mulder (2), in Holland has suggested that Bitter Pit is due to late boron deficiency and internal cork to early boron deficiency. He found that injected borax reduced Bitter Pit from 31% to 4%. Spraying the foliage with 0.25% borax solution reduced Bitter Pit from 68% to 20%.

In view of Mulder's suggestion a field experiment was carried out in 1950 to test the effect of various boron treatments on the incidence of Bitter Pit in the apple var. Grenadier. The boron status of seven samples of apples from varieties showing severe Bitter Pit in 1949, was also determined.

Boron Status of Apples Showing Bitter Pit.

Boron was determined on the dry matter of apple fruits, after ignition, by the method of Berger and Truog (1). The sample for analysis was prepared as follows. The fruits were first washed in tap water, then distilled water and dried. A $\frac{3}{8}$ -inch section was taken from the middle of each fruit at right angles to the stem-eye axis. After removal of the seeds the sections were chopped into small pieces with a stainless steel knife, thoroughly mixed, and sub-samples taken for moisture and boron determinations. The boron status of the 1949 series is given in Table I.

TABLE I.
SYMPTOMS AND BORON STATUS OF APPLES
SHOWING BITTER PIT AND OTHER INTERNAL SYMPTOMS—1949.

Variety	Bitter Pit Symptoms	Date Sampled	Moisture as % Fr. Wt.	Boron as p.p.m. of fruit dry matter
B1. Grenadier	Severe	27/9/49	84.0	15
B2. Newton Wonder	Severe	"	85.2	22
B3. " "	None	"	86.2	24
B4. " "	Severe with internal necrosis	21/9/49	85.2	10
B5. Cox O. P.	Medium	25/9/49	82.8	17
B6. Newton Wonder	Severe with internal necrosis	26/9/49	85.2	10
B7. " "	Medium-severe with some internal flesh necrosis	7/10/49	85.6	21

Although the boron content of B4 and B6 was low, that of B2 was normal, yet all three samples had severe Bitter Pit. Samples B4 and B6 in addition to Bitter Pit showed deep-seated necrotic areas.

Orchard Experiment, 1950.

This orchard of established bush apple trees, var. Grenadier, had shown symptoms of Bitter Pit in previous years, but the symptoms were particularly severe in the very dry season of 1949. In 1950 the following treatments with a four-fold replication were applied on a tree basis.

1. Control trees—no boron.
2. Soil application of borax at the rate of 4 oz. per tree broadcast on 15/3/50.
3. Injection of boric acid on a branch basis, 0.2 to 0.4 g. per branch according to diameter, 15/3/50.
4. Foliage sprays of 0.25% borax + wetting agent. 1st spray applied 12/7/50 : 2nd spray 3/8/50.

The treatments were arranged in four randomised blocks. Injection of boric acid caused localised damage to the bark around the point of injection, but no damage to the foliage. No damage was caused by the foliage sprays.

Samples of fruit were taken for analysis on 9/8/50 and placed in cold store till analysed. At this date no Bitter Pit was showing on the fruits.

On 4 9 50 about 100 apples were picked from each tree and placed in boxes in the orchard store. A count of pitted and normal fruits was made on 22/9/50. The analytical results together with the mean percentage "pitted" fruits (converted to angles for statistical analysis) are given in Table II.

TABLE II.

MEAN BORON CONTENT OF APPLES, VAR. GRENADIER, AND PERCENTAGE OF "PITTED" FRUITS (CONVERTED TO ANGLES) FROM 1950 ORCHARD EXPERIMENT.

	1. Control	2. Soil	3. Injection	4. 2 Sprays	S.E. Mean	Sig. diff. P=0.01
Boron as p.p.m. of fruit dry matter	22.1	28.2	28.6	70.9 *	4.03	18.52
% Bitter Pit converted to angles	21.1	22.3	23.0	24.6	—	N.S.

* Significantly greater than control for P=0.01.

Treatment 4 (foliage spray), was the only one to have any significant effect on the boron status of the fruit. The value 70.9 is much above the usual content of boron in sound fruits. No treatment had any significant effect on the incidence of Bitter Pit.

It would appear from the results of this experiment that the application of boron by means of foliage sprays, although it increased three-fold the boron content of the fruit had no effect on the incidence of Bitter Pit.

Acknowledgments.

We wish to express our thanks to Dr. W. Plant for bringing this problem to our notice and to Mr. P. M. Miller, Harpford Fruit Farm, Sidmouth, for granting facilities for the field experiment and for his help and co-operation.

REFERENCES.

- (1) BERGER, K. C. and TRUOG, E. 1944. Estimation of boron in soils and plants. *Soil Sci.* **57**, 25-36.
- (2) MULDER, D. 1948. *Hortic. Abstracts*, **18**, 1723.
- (3) WALLACE, T., and JONES, J. O. (1940). Boron in relation to Bitter Pit in apples. *J. Pomol.* **18**, 161-176.

THE EFFECT OF ZINC AND COPPER DEFICIENCIES ON CROP PLANTS GROWN IN SAND CULTURE.

By E. J. HEWITT and E. W. JONES.

The importance of zinc as a plant nutrient was recognised as long ago as 1912 by Javillier, but it was only as the result of the careful culture experiments of Mazé (1914) on corn, Sommer and Lipman (1926) on barley and sunflower, Sommer (1928) on buck wheat and beans, Hoagland *et al.* (1938) on fruit trees and of Van Schreven (1937) on potato and sugar beet, that this fact became generally accepted. The widespread incidence and economic importance of zinc deficiency in the field has been recognised in the United States (Chandler, Hoagland and Hibbard, 1931, Camp, 1945), in Australia (Brough 1950), and New Zealand (Forester 1947), and in Hungary, Holland, Denmark and Switzerland (cf. Bould *et al.* 1949), and recently in Great Britain (Bould, Nicholas, Tolhurst, Wallace and Potter, 1949).

The essential status of copper in plant nutrition was established in culture experiments by Sommer (1931), Lipman and Mackinney (1931), Arnon and Stout (1939), and Piper (1942). Copper deficiency in the field is prevalent in cereals, flax, legumes, *Citrus*, apple, pear and other crops. Experiments in Florida, California, S. Africa, Australia and Europe have been described by Floyd (1917), Smith and Thomas (1928), Anderssen (1932), Pittman (1936), Riceman *et al.* (1938, 1940, 1948), Drosdoff and Dickey (1943), Melchers and Gerritsen (1944). Recently a dieback condition of apple trees in Great Britain was attributed to copper deficiency by Bould, Nicholas, Tolhurst, Wallace and Potter (1950).

The work described here was begun to develop an efficient sand culture technique for the production of zinc and copper deficiencies in plants, to record the visual symptoms in economic crops for diagnostic purposes in the field and to investigate further the role of these elements in plant metabolism.

EXPERIMENTAL.

One gallon pyrex glass urn-liners with a central drainage hole at the base or 5-litre pyrex glass beakers of the type described by Hewitt and Jones (1947) were used. These containers were cleaned with a detergent, immersed in concentrated hydrochloric acid, thoroughly "steamed" on the inside for 20 minutes, and finally rinsed with glass distilled water. The sand was similar to that used previously in experiments on molybdenum deficiency by Hewitt and Jones (1947). It was purified as follows in an automatic apparatus described by Hewitt (1947). Each batch was treated with 30 L. of 14%

hydrochloric acid (A.R. Grade), containing 1% oxalic acid (technical grade) for three hours; the apparatus was drained sufficiently to allow 12 L. of concentrated hydrochloric acid and 310 g. of oxalic acid (dissolved in hot tap water) to be added. The sand was then circulated a further three hours in the apparatus, after which it was transferred to a glazed vat from which the free acid was allowed to drain. The sand was leached and "kneaded" with 20 L. of glass distilled water which was then allowed to drain. On the following day the same procedure was repeated but glass distilled water was used instead of tap water for making up the acid mixture. At the end of the "run" the sand was transferred direct from the apparatus to the urns or beakers. The drainage holes were covered by a fine pad of acid extracted glass wool held in place by a pyrex watchglass. Each vessel was leached with several litres of a zinc and copper-free solution of calcium nitrate in glass distilled water at a strength of 16 m.eq. Ca⁺⁺/L. (purified in the manner described below) until the sand reached a pH of 5.

All the water used in the preparation of the nutrient solutions was initially obtained by distillation of softened tap water from copper stills with solid silver condensers, then further distilled from pyrex glass and stored in pyrex glass carboys prior to use.

The following nutrients, in the proportions shown (as m.eq./L.) were applied to each vessel: Ca⁺⁺ 8.0; K⁺ 3.0; Na⁺ 1.33; Mg⁺⁺ 2.2; NO₃⁻ 11.0; PO₄⁻⁻⁻ 4.0; SO₄⁺⁺ 2.2; Fe⁺⁺⁺ (as citrate) 0.3; the following micro-nutrients as p.p.m. were added to the control plants B 0.55; Mn 0.55; Cu 0.064; Zn 0.065; Mo 0.019. Zinc or copper were omitted from the respective deficiency treatments.

The macronutrient reagents were each dissolved in glass distilled water so that the requisite weight for 2 L. of M or 2M solution was contained in approximately 1,900 ml. of solution which was then autoclaved according to the procedure previously outlined by Stout and Arnon (1939), and further modified by Hewitt (1947). The filtrate was made up to 2 litres and subsequently repeatedly extracted with a 0.05% solution of dithizone in re-distilled carbon tetrachloride at least eight times until the green colour of the dithizone layer remained after shaking. Where necessary, prior to the dithizone extraction, the pH of the filtrate was brought to a value of 5.0 with re-distilled hydrochloric acid. On completion of the extraction the stock nutrient solution was allowed to stand in contact with a layer of dithizone in carbon tetrachloride for 4-5 days; it was then decanted into pyrex stock bottles prior to use. Citric acid was re-crystallised from minimal volumes of hot glass distilled water after seeding with minute crystals and stored at 0° C. The crystals were dried with filter paper, an appropriate weight taken, dissolved in glass distilled water and brought to a pH of 5.0 with re-distilled ammonia solution and then repeatedly extracted with dithizone until free of zinc and copper. The zinc and copper-free ammonium citrate was transferred to a silica dish and evaporated down to dryness with constant stirring in the presence of a stoichiometrical amount of zinc and copper-free ferric chloride solution prepared as described previously (Hewitt and Jones, 1949). The ferric citrate was taken up in glass distilled water and used at a concentration of 5.6 p.p.m. Fe in the nutrient as applied to the plants. The micronutrient salts used were all high quality grade salts and were not purified further.

The following crops were grown: Clover (Alsike), Oat (White Star), Sugar Beet (Webb's No. 2), Swede (Perfection Purple Top), Tomato (Market King). All were sown direct in the cultures on 26/6/50 (except clover which

was sown on 10/7/50). The sugar beet and swede seedlings were later thinned out to three plants per vessel and the tomato control treatment reduced to two plants.

Where possible each crop in any particular treatment was grown in both the urn and beaker type of vessel. The type of vessel employed apparently made little difference to the severity of the symptoms obtained and will not be referred to further. The same plants served as controls for the zinc and copper deficiency experiments. With the exception of swede where only one beaker was available, each crop treatment was in duplicate. Prior to planting, each vessel received half a litre of the respective nutrient.

Results.

Visual Symptoms. Zinc deficiency reduced growth and caused a paling or mottling prior to the onset of better defined symptoms. This initial paling was often followed by the occurrence of isolated necrotic areas (oat, sugar beet and tomato) which sometimes were preceded by a well-defined yellow or orange coloured mottle (sugar beet and tomato). Later other effects developed, e.g., undulating or wavy margins of clover and sugar beet foliage; irregular and cupped laminae of swede; and the characteristic coiled or "watch-spring" appearance of the tomato leaves. Flowering was suppressed in clover; ear formation was very weak in oat, and although fruit did set in tomato they were minute. Towards the end of the season (October) when the inrolled nature of the zinc deficient tomato leaves had become marked, examination of the leaflets revealed an accumulation of fluid held in the concavity formed by the lower surface of the lamina. Saprophytic fungi thrived in this fluid including a species of *Pencillium* and *Cladosporium herbarum*.*

Severe and well characterised symptoms of copper deficiency were recorded for both oat and tomato. In oat the well known "white tip" or "reclamation disease" was reproduced. Bleaching with total loss of all pigment (according to visual evidence) tended to occur irregularly in old leaves of beet and swede and in young leaves of oat, and fading was characteristic in clover. These symptoms were in marked contrast to tomato where foliage rapidly developed and maintained a dark blue-green tint. Severe reduction of growth was, however, the most noticeable feature in swede, beet and clover. Copper deficiency drastically reduced the number of flowers formed in clover, prevented ear formation in oat and flower or fruit formation in tomato. Death of the growing point occurred in tomato and in oat. A description of the visual symptoms of zinc or copper deficiency recorded for each plant is given below together with the approximate time in days after sowing (in brackets), necessary for the development of various stages noted.

Zinc Deficiency.

Alsike Clover. Growth drastically reduced. Leaves small, pale to olive green (28 days). Margins of laminae wavy and becoming curled forwards especially at leaf tip, producing "heart-shaped" outline. Localised pale yellow green areas appeared situated between midrib and margin of leaflet; where severe, complete lamina became yellow or orange in colour except for the green bands of tissue adjacent to the midrib (66 days). No flowers formed; shoot elongation suppressed.

Oat. Growth thin; tillering reduced. Leaves initially erect; later

*The writers thank Mr. R. W. Marsh, M.A., for identifying these fungi.

straggling habit. Pale green longitudinal broad striations of lamina (20 days). Chlorotic or practically white areas at base of recently expanded leaves (26 days), followed by dull green, grey or brown water soaked areas similar to an early "grey speck" effect; these became buff coloured or ivory necrotic flecks situated at random along leaf margin and intervenally (30 days). Old leaves developed red, orange or bronze tints especially along the margins (30 days); later becoming brown scorched (39 days), and purple tinted (80 days). Weak ear formation. Free from mildew until partial recovery occurred.

Sugar Beet. Growth diminished and lamina size reduced, habit ultimately rosette. Young and mid stem leaves developed an intervenal paling or yellow green mottle (20 days); the margins became undulating in character (30 days). Brown necrotic areas appeared in the mottled areas initially at random; later concentrated especially at the tip of the leaf in young leaves (61 days). Necrosis spread laterally to give a marginal necrotic scorch (80 days). Necrotic areas became black and yielded a sticky exudate. Intervenal mottling was more marked in older leaves, particularly towards the tip; laminae became narrow in outline, ridged between midrib and margin and bent down (80 days).

Swede. Growth and vigour were greatly reduced. Cotyledons were greener than leaves which showed an intervenal mottling and marginal paling (20 days). Pale dull green foliage (26 days) with orange, yellow or purple mottling in old leaves (39 days) and slight cupping. Marked purple tinting of young leaves. Later necrotic areas appeared in the old leaves beginning at the tip (70 days). Tap root poorly developed.

Tomato. Growth markedly reduced and stunted. Initially darker green than control plants (20 days). Young leaflets mottled yellow green near base and old leaves purple underneath (26 days). Young and old petioles became markedly downcurved; this was more acute in tip than basal leaflets (30 days). Later complete leaf coiled into a "watchspring" shape due to curling under of tip and side leaflets. Dark brown necrotic regions on leaf margin especially obvious on the underside of all leaves (30 days) with exudation of sticky fluid. The necrosis usually appeared first in the older while the mottling was more characteristic in the younger leaves, and was independent of the necrosis. Necrosis spread to veins, base of individual leaflets and along main rachis (39 days); severe necrosis of lamina at leaflet tip and finally complete withering of lamina (80 days). Flowering reduced, few small fruit set. Heights (in cms.) measured after 126 days: Control: 142; -Zn: 72.

Copper Deficiency.

Alsike Clover. Growth severely reduced. Dull green foliage (25 days); leaflets slightly mottled; margins curled upwardly, paling and some scorching (47 days). Lamina distorted by marginal cupping, with slight intervenal chlorosis (66 days). Leaflets finally wilted along margins, scorched and withered (109 days). Flowering practically suppressed.

Oat. Growth and vigour reduced. Bushy habit due to excessive tillering. Leaves initially pale (11 days) with yellow green mottle which disappeared to give a dark green appearance (20 days). Emerging leaves remained rolled and wilted (28 days); white flecking appeared in "kinked" regions of lamina (30 days); later the upper region paled diffusely and became white at the tip and margins (39 days); emerging leaves were later completely white and rudimentary. Oldest leaves faded to bronzed or yellow tint with necrotic brown areas. New lateral shoots were later almost entirely chlorotic and

further leaf emergence ceased due to death of the growing point within (80 days). Ear and grain formation completely suppressed; plants were killed by deficiency.

Sugar Beet. Markedly reduced growth and general stunting. Narrow "thin" strap-like leaves; lustreless pale yellow-green colour (26 days); later a marginal chlorosis of old leaves and irregular completely bleached areas, followed by necrotic marginal scorch and a slight marginal infolding of basal region of old leaves (80 days).

Swede. Growth and vigour severely restricted. Leaves "thin." Initially darker green (20 days) but paling off between veins to give a dull faded green; faintly mottled appearance in middle and old leaves (39 days). Later slight intervenal chlorosis occasionally bleaching and marginal necrosis occurred in the oldest leaves (80 days). Deficient plants free of mildew. No well-defined morphological symptoms observed.

Tomato. Drastic curtailment of growth and vigour. Foliage "thin," dark green (20 days), later distinctive blue-green colour (30 days). Young laminae curled inwardly at the margins towards the upper surface or remained "folded" sharply upwards; petioles became curved downwards (26 days). Cotyledons and older leaves developed irregular brown necrotic areas mainly towards the tips and margins (30 days), especially marked on underside of midrib, and buff coloured in the upper surface of lamina; ultimately completely withered. Young leaves abnormally narrow and straplike; (and sharply turned upwards along margins), extreme reduction in lamina area (39 days). Middle leaves curved downwards but margins still upwardly curled. Old leaves completely withered (123 days). Cotyledons tinged with purple along margins (80 days). No flowers produced. Growing point of some plants killed (26 days) and (30 days). Heights (in cms.), measured after 126 days:—Control: 142; -Cu: 22.

Yields.

The mean dry and fresh weights recorded for shoot, root and complete plant for each treatment are given in Table I. Growth of the control oat plants was checked to a considerable extent owing to mildew and the growth of deficient plants in terms of per cent. of the control treatment was accordingly high.

Copper deficiency reduced growth as much as and usually more than zinc deficiency. The tomato was outstandingly more sensitive to copper deficiency than the others. The order of decrease of yield (as dry weight per cent. of control) were:—

-Zn : Clover, Swede, Tomato, Beet, Oat.

-Cu : Tomato, Swede, Clover, Beet, Oat.

Although copper deficiency generally had a more severe effect than zinc on the reduction of total growth it was found that the shoot to root ratio (cf. Table I) was generally higher for the copper than for the zinc deficiency treatments on both dry and fresh weight bases. On the fresh weight basis it would seem that the absence of either zinc or copper from the medium deterred root development—slightly in the case of zinc but markedly so with copper. A similar but not so consistent trend was obvious on the basis of the dry weight results. In addition to the proportion of root produced the absolute amount of root produced was usually smaller in the copper than in the zinc deficient plants; this was especially true of the sugar beet. Examples of effects described above are shown in Plate III, Figs. 1 and 2.

TABLE I.
FRESH AND DRY WEIGHT YIELD DATA
FOR PLANTS GROWN UNDER CONDITIONS OF ZINC AND COPPER DEFICIENCY.

Crop	Dry Weight g./Plant			Fresh Weight g./Plant		
	Control	-Zn	-Cu	Control	-Zn	-Cu
<i>Tomato.</i>						
Shoot & Fruit ..	141.7	14.0	1.25	1538	103.3	8.50
Root ..	14.3	5.45	0.26	82	36.5	2.00
Complete Plant ..	156	19.45	1.51	1620	139.8	10.50
% of Control ..	—	12.45	0.97	—	8.63	0.65
Shoot/Root ..	9.9	2.6	4.8	18.8	2.8	4.3
<i>Oat.</i>						
Shoot ..	5.85	2.65	1.17	—	—	—
Root ..	1.95	0.95	0.71	—	—	—
Complete Plant ..	7.80	3.60	1.88	—	—	—
% of Control ..	—	46.2	24.1	—	—	—
Shoot/Root ..	3.0	2.8	1.6	—	—	—
<i>Clover.</i>						
Shoot ..	15.5	0.61	1.01	70.5	2.82	6.11
Root ..	21.0	0.97	0.97	147.5	7.74	7.59
Complete Plant ..	36.5	1.58	1.98	218.0	10.56	13.70
% of Control ..	—	4.33	5.42	—	4.84	6.28
Shoot/Root ..	0.74	0.63	1.0	0.48	0.36	0.81
<i>Sugar Beet.</i>						
Shoot ..	30.42	12.01	4.54	230	72.75	35.33
Root ..	73.38	20.11	2.53	284.4	73.15	12.17
Complete Plant ..	103.8	32.12	7.07	514.4	145.9	47.50
% of Control ..	—	31.0	6.82	—	28.40	9.23
Shoot/Root ..	0.42	0.60	1.79	0.81	0.99	2.90
<i>Swede.</i>						
Shoot ..	21.5	1.05	1.98	161.5	6.1	15.05
Root ..	86.0	4.0	2.9	715.5	24.0	8.8
Complete Plant ..	107.5	5.05	4.88	877	30.10	23.85
% of Control ..	—	4.70	4.54	—	3.44	2.72
Shoot/Root ..	0.25	0.26	0.68	0.23	0.25	1.71

DISCUSSION.

The results of the experiment indicated that the technique employed was mainly successful, but there is scope for improvement in the efficiency of the purification processes generally. Occasional recovery was seen in some crops, especially tomato and oat in the zinc deficiency treatments, and the proportion of the deficient to control yields was greater than anticipated for these and for sugar beet. Severe mildew attack on the oat was contributory in this crop especially as the zinc deficient plants remained "clean" until partial recovery occurred when mildew rapidly developed. The zinc content of the glass distilled water was probably about 5 $\mu\text{g./L.}$ and as redistillation caused little further reduction according to analytical tests this point will be investigated in greater detail. Both yield and visual data suggested that the purification techniques usually were more efficient in the removal of copper than of zinc.

Different crops had markedly different requirements for either of these micronutrients. Sugar beet produced some of the most diagnostic symptoms of zinc deficiency, yet on the basis of yield, produced a far better crop under those conditions than any of the other plants. This might indicate that here

the effect of zinc deficiency on assimilation was less than on some other aspect of metabolism responsible for visual symptoms. Swede, however, did not exhibit such well defined visual symptoms yet the relative severity of the deficiency was greater than in sugar beet.

Most of the crops appeared equally susceptible to copper deficiency on a yield basis (with the possible exception of oat), but as in zinc deficiency, certain plants (e.g. oat and tomato) were characterised by well defined visual symptoms and others (e.g. sugar beet and swede) mainly by retarded growth.

Both zinc and copper deficiency (with the exception of tomato) caused fading or sometimes a distinct chlorosis subsequently followed by a necrosis. Camp (1945) has suggested that zinc is related to chlorophyll formation in some undetermined way, and Sommer (1945) has discussed the evidence in support of the direct catalytic effect of copper on chlorophyll formation. The observations recorded above suggested that stability of chlorophyll might be concerned. The rosetting and morphological deformations of the leaves induced under conditions of zinc deficiency in the above experiment were consistent with its relation to auxin formation and water relations as suggested by Skoog (1940) and Tsui (1948), and analogous to the effects recorded in other plants.

Zinc and sometimes copper deficiencies caused distinct purple tinting reminiscent of phosphate deficiency. These observations agreed with those made by Reed (1946) who found that zinc deficient tomato plants contained more inorganic but less organically bound phosphate than normal plants. He concluded that a phosphorase enzyme system was inhibited by zinc deficiency.

The sticky brown leaf exudation observed with zinc deficiency might indicate breakdown of the cell membranes. This would be consistent with the observations of Reed (1941) (1946), on the effect of zinc deficiency on cell organisation and on phosphorus metabolism where the energy relations of the cell are probably involved.

Acknowledgments.

This work was carried out with the aid of special grants from the Agricultural Research Council whose support is gratefully acknowledged. We wish to thank also Mr. G. H. Jones, A.R.P.S., for the photographic records of the plants.

Summary.

1. Clover, oat, sugar beet, swede and tomato were grown in zinc and copper deficient sand cultures.
2. Methods for nutrient reagent purification and the preparation of purified sand and water supplied are described.
3. Clover, oat, sugar beet and tomato showed acute visual symptoms of zinc deficiency; swede showed less well defined symptoms but growth was greatly reduced. Yields were reduced between 4.3 and 46.2 per cent. of control plants as expressed on a dry weight basis in the increasing order as follows: clover, swede, tomato, sugar beet and oat. Tomato and sugar beet were the best visual indicators of zinc deficiency.
5. Visual symptoms of copper deficiency were acute in tomato and oat, and less marked in clover, sugar beet and swede. Yields were sharply reduced between 0.97 and 24.1 per cent. of control plants as expressed on a dry weight basis in increasing order of susceptibility

PLATE III.



Fig. 1. Effect of zinc deficiency on tomato foliage.
Note rolling under of leaflets and marginal or tip necrosis.



Fig. 2. Effect of copper deficiency (right) on growth of tomato.

as follows: tomato, swede, clover, sugar beet and oat; oat and tomato were good visual indicators of copper deficiency.

REFERENCES.

- ANDERSSON, F. G. (1932). Chlorosis of deciduous fruit trees due to a copper deficiency. *J. Pom. and Hort. Sci.*, **10**, 130.
- ARNON, D. I. and STOUT, P. R. (1939). The essentiality of certain elements in minute quantity for plants with special reference to copper. *Plant Physiol.*, **14**, 371.
- BOULD, C., NICHOLAS, D. J. D., TOLHURST, J. A. H., WALLACE, T. and POTTER, J. M. S. (1949). Zinc deficiency of fruit trees in Britain. *Nature*, **164**, 801.
- BOULD, C., NICHOLAS, D. J. D., TOLHURST, J. A. H., WALLACE, T. and POTTER, J. M. S. (1950). Copper deficiency of fruit trees in Britain. *Nature*, **165**, 720.
- BROUGH, C. R. (1950). Zinc deficiency in deciduous fruit trees in Victoria. *J. Dept. Agr. Victoria*, **48**, 257.
- CAMP, A. F. (1945). Zinc as a nutrient in plant growth. *Soil Sci.*, **60**, 157.
- CHANDLER, W. H., HOAGLAND, D. R. and HIBBARD, P. L. (1931). Little leaf or rosette in fruit trees. *Proc. Amer. Soc. Hort. Sci.*, **28**, 556.
- DROSDOFF, M. and DICKEY, R. D. (1943). Copper deficiency of Tung trees. *Proc. Amer. Soc. Hort. Sci.*, **42**, 79.
- FLOYD, B. F. (1917). Dieback or exanthema of *Citrus* trees. *Bull. Florida Agr. Exp. Sta. No. 140*.
- FORESTER, E. D. (1947). Zinc deficiency in New Zealand apple trees. *Orchardist of N. Zealand*, 20(3) 4-5.
- HEWITT, E. J. (1947). A technique for large scale pot sand cultures. *Ann. Rep. Long Ashton Res. Sta.*, 1946, p. 37.
- HEWITT, E. J. and JONES, E. W. (1947). The production of molybdenum deficiency in plants in sand culture with special reference to tomato and Brassica crops. *J. Pom. and Hort. Sci.*, **23**, 254.
- HEWITT, E. J. and JONES, E. W. (1949). Molybdenum as a plant nutrient. The effects of molybdenum deficiency on some vegetables, cereals and forage crops. *Ann. Rep. Long Ashton Res. Sta.*, 1948, p. 81.
- HOAGLAND, D. R., CHANDLER, W. H. and HIBBARD, P. L. (1935). Little leaf or rosette of fruit trees. V. Effect of zinc on the growth of plants of various types in controlled soil and water culture experiments. *Proc. Amer. Soc.*, **33**, 131.
- HOAGLAND, D. R. and STOUT, P. R. (1936). Little leaf or rosette of fruit trees. VI. Further experiments bearing on the cause of the disease. *Proc. Amer. Soc. Sci.*, **34**, 210.
- JAVILLIER, M. (1912). Sur l'emploi du zinc comme engrais catalytique. *Internatl. Cong. Appl. Chem. Orig. Commun.*, **15**, 145.
- LIPMAN, C. B. and MACKINNEY, G. (1931). Proof of the essential nature of copper for the higher green plants. *Plant Physiol.*, **6**, 593.
- MAZÉ, P. (1914). Influences respectives des elements de la solution minerale sur le development du maïs. *Ann. Inst. Pasteur*, **28**, 21.
- MELCHERS, W. J. and GERRITSEN, H. J. (1944). "Koper als omnisbar element voor plant endier." (Wageningen).
- PIPER, C. S. (1942). Investigations on copper deficiency in plants. *J. Agr. Sci.*, **32**, 143.
- PITTMAN, H. A. (1936). *J. Dep. Agric. W. Aust. II*, **13**, 187. Cited by Bould *et al.* (1950).
- REED, H. S. (1941). Effects of zinc deficiency on cells of vegetative buds. *Amer. J. Bot.*, **28**, 10.
- REED, H. S. (1946). Effects of zinc deficiency on the phosphate metabolism of the tomato plant. *Amer. J. Bot.*, **33**, 778.
- RICEMAN, D. S. and DONALD, C. M. (1938). Copper deficiency in plants at Robe, South Australia. *C.S.I.R. Australia, Pamphlet No. 78*.
- RICEMAN, D. S., DONALD, C. M. and STEVENS, S. T. (1940). Further investigations in copper deficiency in plants in South Australia. *C.S.I.R. Australia, Pamphlet No. 96*.
- RICEMAN, D. S. (1948). Mineral deficiency in plants on the soils of the Ninety Mile Plain in South Australia. 2. Effect of zinc, copper and phosphate or subterranean clover and lucerne grown in Laffer sand, near Keith. *C.S.I.R. Australia, Bulletin No. 234*.
- SKOOG, F. (1940). Relationships between zinc and auxin in the growth of higher plants. *Amer. J. Bot.*, **27**, 939.
- SMITH, R. E. and THOMAS, H. E. (1928). Copper sulphate as a remedy for exanthema in prunes, apples, pears and olives. *Phytopath.*, **18**, 449.
- TSUI, CHENG (1948). The effect of zinc on water relation and osmotic pressure of the tomato plant. *Am. J. Bot.*, **35**, pp. 172, 309.
- SOMMER, A. L. (1928). Further evidence of the essential nature of zinc for the growth of higher green plants. *Plant Physiol.*, **3**, 217.
- SOMMER, A. L. (1931). Copper as an essential to plant growth. *Plant Physiol.*, **6**, 339.
- and LIPMAN, C. B. (1926). Evidence of the indispensable nature of boron and zinc for higher plants. *Plant Physiol.*, **1**, 231.
- STOUT, P. R. and ARNON, D. I. (1939). Experimental methods for the study of the role of copper, manganese and zinc in the nutrition of the higher plants. *Amer. J. Bot.*, **26**, 144.
- VAN SCHREVEN, D. A. (1937). Zinc as an essential element for sugar beet and potato plants. *Meded. Inst. voor Suikerbieteneteelt*, **7**, 1.

EXPERIMENTS ON IRON METABOLISM IN PLANTS.

III. THE RELATION OF MOLYBDENUM AND NITROGEN SUPPLY TO METAL-INDUCED IRON DEFICIENCY IN SUGAR BEET.

By E. J. HEWITT.

This paper gives results of further work on the inter-relationship between heavy metals and the role of iron in chlorophyll formation. It was shown by Hewitt (1949) that extra molybdenum (at 0.6 m.eq./L. Mo^{VI}) accentuated the iron-deficiency chlorosis induced by copper, zinc, manganese and trivalent chromium. The great response to molybdenum suggested that some aspect of nitrogen metabolism might be involved. A preliminary trial in 1948 where urea was given in place of nitrate was inconclusive. A quantitative experiment was made in 1949 to test the effect on sugar beet of using either nitrate or urea at normal or high levels of molybdenum, with or without excess of a number of heavy metals.

EXPERIMENTAL.

1949 Experiment.

Methods.

The general technique was similar to that adopted previously. Nitrate was given at 8 m.eq./L., or urea at an equivalent rate of N. Iron was supplied at 0.075 m.eq./L. as ferric citrate. Molybdenum was given at 0.0012 or 0.3 m.eq./L. There were thus four basal series of solutions. Each of these was used alone or with the addition of 0.3 m.eq./L. Cr^{+++} , CrO_4^{--} , Mn^{++} , Co^{++} , Cu^{++} , Zn^{++} or Cd^{++} and made 32 factorial treatments in all. They were replicated in two randomised blocks with split plot layout for level of molybdenum.

Results.

Chlorosis ranged from nil to severe according to treatment, and the general effects were summarised as follows:—

<i>NO₃ Series.</i>	<i>Normal Mo.</i>		<i>High Mo.</i>
Severe	Cd, Co, Cu	Severe	Cd, Co, Cu
Moderate	CrO_4	Moderate to severe	Mn, Cr, Zn
Slight	Zn, Cr	Slight to moderate	CrO_4
Nil	Mn		

<i>Urea Series.</i>	<i>Normal Mo.</i>		<i>High Mo.</i>
Severe	Cd, Co, Cu, CrO_4	Severe	All metals
Moderate	—		
Slight	Zn, Cr		
Nil	Mn		

Nitrate Series.

The great "activity" (in terms of chlorosis produced) of cadmium, noted in 1948, was confirmed. The effects with cadmium and cobalt were too severe to detect any clear interaction with molybdenum although there was slight

indication of this with copper. The general order of activity noted in the previous experiments (Hewitt 1949) was confirmed. Molybdenum again greatly accentuated chlorosis caused by manganese, zinc and trivalent chromium. Chlorosis in the presence of chromate was *reduced* by extra molybdenum.

The toxicity symptoms as distinct from chlorosis resulting from presence of the heavy metals were described by Hewitt (1949) and appeared in the plants grown in this experiment.

Urea Series.

The use of urea in place of nitrate had interesting results. Growth was somewhat reduced but not markedly so.

Old leaves contained more chlorophyll than those of plants in the nitrate series. The severity and pattern of chlorosis in the presence of certain heavy metals was modified. Chromate induced almost complete chlorosis in the presence of urea and the contrast with nitrate was particularly striking. Extra molybdenum did not reduce the chlorosis in this treatment. Zinc, manganese and trivalent chromium resulted in slight speckling of the younger and middle leaves. This was not typical of iron deficiency as the leaves were dark green and the intervenal areas were diffuse and irregularly yellow green. Extra molybdenum greatly accentuated chlorosis with these metals and plants were as severely chlorotic as those given cadmium, copper and cobalt where no molybdenum interaction was detected. The general extent of chlorosis exceeded that in the nitrate series. Examples of the results recorded here are shown in Plate No. IV.

Chlorophyll content. The visual observations were checked by determination of total chlorophyll in old and young leaves at intervals. The method will be described in detail elsewhere*. It consisted in homogenising $\frac{1}{2}$ -1 g. samples comprising fragments from several fresh leaves, with pyrex glass or emery powder in 85% acetone for 1-2 minutes. The filtered extracts were made up to 50 ml. with 85% acetone and "Spekkered." A calibration curve based on dilution of a standard chlorophyll solution was used to estimate the concentration. All extracts were kept out of light during preparation and in a refrigerator until measured. The yellow solution of carotene pigments apparently did not interfere appreciably as shown by the close approach of readings for entirely yellow leaves to "blank" determinations.

The results are given in Table I. Limited statistical analyses shown in Table II indicated that most of the differences were significant. The data confirmed the visual observations including the effects of molybdenum on chlorosis induced by chromate in the presence of nitrate. The effect of molybdenum was at least as great with urea as with nitrate.

Urea resulted in a higher chlorophyll concentration in old leaves. The reduction in chlorophyll content in young leaves due to molybdenum was consistently greater with urea than with nitrate and the difference between urea and nitrate was greater in the presence of molybdenum. These values did not approach significance however, although their existence had been inferred from the visual appearance of the plants.

Yields and Percent Dry Weight. The mean total dry weight yields are also given in Table I. Urea was slightly toxic to growth but growth was severely reduced by the unfavourable combinations of certain heavy metals,

* The method was evolved with the collaboration of Mr. E. W. Jones as a quick procedure for use with numerous samples.

TABLE I.

EFFECT OF HEAVY METALS, MOLYBDENUM AND NITROGEN SUPPLY ON YIELD, PERCENT DRY WEIGHT, AND CHLOROPHYLL PRODUCTION IN SUGAR BEET.

Treatment	Mean Yield and Mortality			Mean Percentage Dry Wt.			Mean Chlorophyll Content mgs./100 g. Fresh Wt.					
	Mean Fresh Wt.	Mean Dry Wt.	Mean No Killed	Young Leaf- July	Old Leaf- July	Tap Root- Oct.	Young Leaves			Old Leaves		
							July	Aug.	Oct.	July	Aug.	Oct.
Nitrate Normal Mo Basal	2164.5	476.75	0	19.98	13.13	22.15	158.40	132.15	186.60	89.1	75.20	137.35
" " +CrO ₄	1593	280.35	0	15.85	12.00	20.85	70.85	31.40	35.90	55.00	44.15	51.95
" " +Cr+++	1819	359.25	0	19.50	10.95	21.60	146.00	110.40	131.25	71.70	61.35	89.50
" " +Mn++	1926	398.05	0	19.48	12.83	22.60	138.75	119.25	141.00	76.45	69.90	96.15
" " +Co++	168.5	21.3	6	24.55	17.00	14.15	7.90	17.60	10.40	80.75	26.40	27.2
" " +Zn++	564.5	99.8	2.5	17.58	15.08	17.38	28.20	7.45	4.90	80.35	26.40	8.80
" " +Cu++	1870	370.75	0	17.73	13.33	21.45	103.60	112.55	152.15	66.80	81.30	100.65
" " +Cd++	318.5	52.95	7	12.53	13.68	12.68	5.60	10.50	7.05	34.25	20.50	8.65
Nitrate High Mo Basal	1954.5	396.9	0	19.68	13.08	22.75	150.85	86.6	100.90	86.15	63.60	83.40
" " +CrO ₄	1611	322.05	0	18.80	12.50	21.80	136.55	45.2	25.60	74.10	53.30	46.50
" " +Cr+++	1699	305.1	.5	17.68	11.80	19.93	95.00	25.9	32.35	72.15	63.10	44.15
" " +Mn++	1622.5	274.75	.5	18.40	10.65	18.83	52.85	31.35	17.25	66.65	49.15	35.50
" " +Co++	150	21.8	9	22.45	17.68	13.80	9.95	11.00	12.20	70.45	22.55	—
" " +Cu++	215.5	31.2	12	16.5	16.63	10.93	10.20	3.70	1.70	83.50	27.25	—
" " +Zn++	1380.5	243.75	0	16.25	13.95	20.05	46.75	46.15	38.20	49.30	34.15	38.80
" " +Cd++	305.5	47.0	4	14.1	14.03	12.33	14.30	16.10	5.65	51.00	15.70	5.80
Urea Normal Mo Basal	1245.5	245.45	0	19.03	16.23	22.30	110.1	112.75	163.75	124.25	86.70	116.00
" " +CrO ₄	414	71.35	2.5	15.20	16.20	18.10	17.5	16.80	19.15	72.10	33.75	21.65
" " +Cr+++	1163	211.95	0	18.90	16.08	22.00	84.15	86.30	120.30	116.80	80.15	88.25
" " +Mn++	1032.5	193.85	1	17.40	15.58	20.60	77.65	71.45	153.05	101.65	70.40	103.90
" " +Co++	77.5	14.15	9	26.25	21.35	14.33	9.55	13.75	11.80	61.80	21.95	—
" " +Zn++	183.5	33.45	8	15.45	15.43	16.68	10.95	7.60	19.20	83.65	31.15	20.8
" " +Cu++	1183	217.6	0	19.25	18.43	20.25	94.25	85.45	136.40	112.10	78.90	102.00
" " +Cd++	99	15.7	9	13.83	16.73	13.25	4.15	7.15	8.60	60.00	16.10	—
Urea High Mo Basal	1072	205.7	0	18.70	16.53	22.15	99.85	98.90	74.40	137.50	82.25	80.70
" " +CrO ₄	243.5	43.75	6.5	17.53	15.53	16.68	14.15	2.45	12.85	74.80	34.25	22.00
" " +Cr+++	347	56.2	4	15.90	13.3	13.75	31.95	14.15	8.95	92.40	38.00	8.65
" " +Mn++	358	58.25	6	15.05	14.13	14.65	20.05	13.40	7.65	77.80	36.75	20.25
" " +Co++	103.5	15.2	5	23.80	20.70	13.65	8.70	13.80	19.65	73.15	28.3	—
" " +Zn++	247.5	48.45	4	16.55	16.55	20.20	13.90	7.60	5.80	102.65	44.65	6.2
" " +Cu++	368	65.0	4	16.55	15.30	16.70	34.00	22.55	15.05	70.30	39.25	41.65
" " +Cd++	111.5	18.85	7.5	15.75	17.80	13.00	3.90	6.55	8.50	57.30	17.75	5.8

TABLE II.
HEAVY METALS EXPERIMENT: CHLOROPHYLL MG/100 GM OF FRESH WEIGHT.
SUGAR BEET, 1949.
JULY (YOUNG LEAVES).

—	Basal	CrO ₄	Cr	Mn	Co	Cu	Zn	Cd	Mean
NO ₃	154.6	103.7	120.5	95.8	8.9	19.8	75.2	10.0	73.6
Urea	105.0	15.8	58.8	48.8	9.1	12.4	64.1	4.0	39.8
								(±3.20)	
Normal Mo	134.2	44.2	115.1	108.2	8.7	20.1	98.9	4.9	66.8
High Mo	125.4	75.4	64.2	36.4	9.3	12.1	40.4	9.1	46.5
Mean	129.8	59.8	89.6	72.3	9.0	16.1	69.6	7.0	56.7
				(±4.53)					

S.E. for vertical comparisons ±9.05.

S.E. for horizontal comparisons ±11.09.

JULY (OLD LEAVES)

—	Basal	CrO ₄	Cr	Mn	Co	Cu	Zn	Cd	Mean
NO ₃	87.6	64.6	72.4	71.6	75.6	81.9	58.0	42.6	69.3
Urea	130.9	73.4	104.6	89.7	67.5	93.2	91.2	58.6	88.6
								(±2.43)	
Normal Mo	106.7	63.6	94.8	89.0	71.3	82.0	89.4	47.1	80.5
High Mo	111.8	74.4	82.3	72.2	71.8	93.1	59.8	54.2	77.4
Mean	109.2	69.0	88.5	80.6	71.6	87.6	74.6	50.6	79.0
				(±4.68)					

S.E. for vertical comparisons ±6.86.

S.E. for horizontal comparisons ±9.53.

JULY (YOUNG LEAVES)

	NO ₃	Urea	Mean
Normal Mo	82.5	51.0	66.8
High Mo	64.6	28.5	46.5 (±3.20)
Mean	73.6	39.8	56.7
	(±3.20)		

S.E. for horizontal and vertical comparisons ±4.53.

S.E. for diagonal comparisons ±5.54.

(OLD LEAVES)

	NO ₃	Urea	Mean
Normal Mo	69.4	91.5	80.4
High Mo	69.2	85.7	77.4 ±(2.43)
Mean	69.3	88.6	79.0
	(±2.43)		

S.E. for horizontal and vertical comparisons ±(3.43).

S.E. for diagonal comparisons ±(4.77).

molybdenum and nitrogen source. Percent dry weight in leaves and tap root was usually reduced by heavy metal treatments. Cobalt was exceptional and caused increased dry weight in young and old leaves in July. Urea increased the dry weight of old leaves, reduced it in young leaves and did not affect it in the tap root. Statistical tests showed the main effects were significant.

Iron Content. Fresh leaves and roots were homogenised at intervals in reagents such as 0.1N.HCl and ether or N. glycerophosphoric acid or frozen in acetone/solid carbon dioxide and pressed. The extracts were analysed for iron. As no consistent results were obtained and no clear trends could be discerned the results are not given here. Total iron determinations have not been completed. The use of the reagents mentioned has been studied in connection with other work on problems of iron nutrition in collaboration with Mr. E. W. Jones.

1950 Experiment.

Methods.

The general technique was similar to that described above. Owing, however, to unforeseen difficulties, spinach beet had to be used in place of sugar beet and the work was started late. The object of the experiment was to repeat the 1949 trial with ammonium nitrogen in place of urea. The effects of giving iron as ferric citrate or ferrous sulphate (at 0.075 m.eq. Fe^{+++} or 0.05 m.eq. Fe^{++} /L.) were also tested. The heavy metals tested were limited to the essential elements manganese, zinc and copper, each at 0.2–0.5 m.eq./L. The high molybdenum level was 0.3 m.eq./L. There were thus 32 factorial treatments as before.

Results.

The heavy metal concentrations selected as suitable for sugar beet were not so well matched to the sensitivity of spinach beet, which was somewhat less susceptible. The late start prevented proper adjustment in the levels in time. For these reasons results will not be given in detail, but some responses were sufficiently pronounced to be recorded here.

Copper (as before) was the most active of the three metals in causing chlorosis in the presence of nitrate, and ferric iron. Its effects greatly exceeded those of zinc or manganese. The interaction of extra molybdenum was seen with each metal but was less pronounced than in sugar beet, except that the reduced sensitivity of the spinach beet allowed the effect with copper to be seen quite clearly.

Ammonium sulphate proved highly toxic and growth in this series was greatly reduced. In spite of this result it was observed that manganese induced considerable chlorosis which did not occur when nitrate was given, or with ammonia only. The responses to zinc or copper were not markedly different from those when nitrate was given. There was possibly a slight increase in chlorosis in the presence of zinc but the reduced growth might have partly accounted for such an effect.

The response to extra molybdenum observed with nitrate was not pronounced with ammonium sulphate contrary to that with urea in the sugar beet experiment. The source of iron supply did not materially affect results, except possibly in the presence of copper. Here ferrous sulphate seemed to be more effectively utilised than ferric citrate.

DISCUSSION.

The results presented here confirm those already reported. Extra molybdenum consistently accentuated chlorosis in the presence of manganese, trivalent chromium, zinc and copper, contrary to the original findings of Millikan (1947) with flax in water cultures.

Results with chromium salts were especially interesting. There appeared to be a specific relation between source of nitrogen (as urea), and the degree of chlorosis induced by chromate, but not by chromic sulphate. The influence of molybdenum was also reversed when chromate was given as compared with chromic sulphate. Discussion of this aspect of the problem would be premature at present but the nature of the exception seems to emphasise the importance of nitrogen and molybdenum nutrition in iron metabolism, especially when heavy metals are present.

The increased chlorophyll content of old leaves in the presence of urea, and the accentuated chlorosis of younger foliage when heavy metals were also given probably accounted for the inconclusive results obtained in 1948. Possibly two contrasting effects were involved, one tending to increase overall production of chlorophyll, the other accentuating the interference with that aspect of iron nutrition under the unfavourable circumstances due to presence of heavy metals.

The results for the 1950 experiment provide little scope at present for discussion. The effect of manganese in the presence of ammonium sulphate provided further evidence of the importance of nitrogen supply in the utilisation of iron for chlorophyll formation, and also of the relationship between manganese and nitrogen metabolism under conditions of deficiency or excess as noted already by Arnon (1938), Hewitt, Jones and Williams (1948), and Hewitt and Jones (unpublished).

The fact that molybdenum accentuated chlorosis was thought at first to suggest that stimulation of nitrate reduction was a possible factor. The observation that molybdenum exerts the same effect when nitrogen other than nitrate is given suggests molybdenum exerts its influence through some system that is *independent* of source of nitrogen. Whether nitrate reduction depends on such a system in an incidental manner, and whether molybdenum is involved in some other aspect of metabolism concerned directly with iron nutrition may become clear as this work progresses. The "triangular" relationships between manganese or other heavy metals, iron and molybdenum, and between manganese, iron and nitrogen supply and the known effects of molybdenum on nitrate assimilation suggest, however, that the problem embraces all these aspects of metabolism in a closely interrelated system.

Acknowledgments.

The statistical examination of chlorophyll and yield data was undertaken by the Agricultural Statistical Service at Rothamsted Experimental Station, to whom grateful acknowledgment is made for their assistance.

Summary.

- (1) Experiments to test the effect of Cr^{+++} , CrO_4^{--} , Mn^{++} , Co^{++} , Cu^{++} , Zn^{++} and Cd^{++} on chlorophyll production in sugar beet were continued, with special reference to their inter-relationships with molybdenum and nitrogen supply.

- (2) Cd^{++} , Cu^{++} , and Co^{++} caused severe, and CrO_4^{--} moderate chlorosis. Zn^{++} , Cr^{+++} and Mn^{++} only caused slight chlorosis in decreasing order, when given with nitrate nitrogen.
- (3) Extra molybdenum in the presence of nitrate increased chlorosis caused by Mn^{++} , Cr^{+++} , Zn^{++} and Cu^{++} . There was no significant response with Co^{++} and Cd^{++} ; chlorosis with CrO_4^{--} was reduced by molybdenum.
- (4) Nitrogen as urea caused a great increase in chlorosis due to CrO_4^{--} . Effects with other metals generally resembled those seen with nitrate. Urea also caused more chlorophyll to appear in old leaves but in the presence of extra molybdenum metal-induced chlorosis in young leaves was more severe than with nitrate.
- (5) The effects of molybdenum on chlorosis in spinach beet caused by Mn^{++} , Zn^{++} and Cu^{++} in the presence of nitrate, were similar to those in sugar beet. Ammonium nitrogen greatly increased chlorosis caused by Mn^{++} . Effect of ammonium nitrogen was generally toxic and other interactions could not be detected. Effect of valency of iron was inconclusive.
- (6) The general effects of chlorosis were confirmed by chlorophyll determinations and yields, all of which were significant.

REFERENCES.

- ARNON, D. I. (1937). Ammonium and nitrate nitrogen nutrition of barley at different seasons in relation to hydrogen ion concentration, manganese, copper and oxygen supply. *Soil Sci.*, **44**; 91.
- HEWITT, E. J. (1949). Experiments on iron metabolism in plants. I. Some effects of metal induced iron deficiency. *Ann. Rep. Long Ashton Res. Stn.* 1948 p.66.
- HEWITT, E. J., JONES, E. W. and WILLIAMS (1949). The relation of molybdenum and manganese to the free amino acid content of cauliflower plants in sand culture. *Nature*, **163**; 681.
- MILLIKAN, C. R. (1947). Effect of molybdenum on the severity of toxicity symptoms in flax induced by an excess of either manganese, zinc, copper, nickel or cobalt in the nutrient solution. *J. Austral. Inst. Agr. Sci.*, **13**; 180.

PLATE IV.



Iron nutrition (Heavy metals). Effects of sodium chromate on sugar beet in relation to molybdenum and nitrogen supply.

Left to right :—Nitrate (Normal Mo), Nitrate (High Mo), Urea (Normal Mo) and Urea (High Mo).

Note.—Molybdenum reduces chlorosis with chromate in the presence of nitrate; urea or urea and molybdenum increase chlorosis.



Iron nutrition (Heavy metals). Effects of chromic sulphate on sugar beet in relation to molybdenum and nitrogen supply.

Left to right :—Nitrate (Normal Mo), Nitrate (High Mo), Urea (Normal Mo) and Urea (High Mo).

Note effect of molybdenum in increasing chlorosis with nitrate or urea.

EXPERIMENTS ON IRON METABOLISM IN PLANTS.

IV. THE INTER-RELATIONSHIP OF IRON AND POTASSIUM IN THE POTATO AS AFFECTED BY THE PRESENCE OF CALCIUM CARBONATE.

By E. W. JONES.

A preliminary paper in this series (4) described some experimental results which supported the idea that iron and potassium were inter-related in the metabolism of the potato plant. The present paper reports results obtained from a factorial experiment in which this inter-relationship was further investigated and confirmed both in the presence and absence of calcium carbonate.

Experimental.

Four levels of iron were given at each of four levels of potassium with, or without calcium carbonate. The iron and potassium levels were chosen to represent severe deficiency, mild deficiency, normal and luxury consumption. These were (in terms of milliequivalents per litre) $\text{Fe}_1 = 0.0005$; $\text{Fe}_2 = 0.0075$; $\text{Fe}_3 = 0.113$; $\text{Fe}_4 = 1.69$; $\text{K}_1 = 0.50$; $\text{K}_2 = 1.25$; $\text{K}_3 = 3.13$; $\text{K}_4 = 7.81$.

The remaining nutrient elements were added in the following concentrations (expressed as milliequivalents per litre):— $\text{Ca}^{++} 8.0$; $\text{Na}^+ 6.33$ (K_1 , K_2 and K_3), 1.33 (K_4); $\text{Mg}^{++} 3.0$; $\text{PO}_4^{---} 3.0$; $\text{NO}_3^- 13.0$; $\text{SO}_4^{--} 3.49$ (K_1); 4.21 (K_2); 6.04 (K_3), 5.66 (K_4); $\text{Mn}^{++} 0.02$; $\text{Cu}^{++} 0.002$; $\text{Zn}^{++} 0.002$; $\text{BO}_3^{--} 0.09$; $\text{Mo}^{\text{VI}} 0.0012$ (as ammonium molybdate). Thus each treatment received a supply of soluble calcium. In addition, one-half of the pots (C_2 treatments) each received 50 grams of A.R. calcium carbonate which was well mixed with the sand ($9\frac{1}{2}$ kilos per pot) prior to planting; treatments not receiving calcium carbonate were designated C_1 . The nutrient and sand purification techniques were similar to those described elsewhere (3). Certified Scotch seed tubers, variety Majestic, weighing between 30 and 80 g. were washed and planted on 10/5/48. "Young" and "old" leaves were sampled in June, July, August and September for analysis. Chlorophyll was determined in 85% acetone by the method of Comar *et al.* (1, 2). "Soluble iron" was determined after extraction either with 0.1 N hydrochloric acid saturated with ether or with 3.4% glycerophosphoric acid; (both free from iron). The laminae were washed either with glass distilled water or weak hydrochloric acid and ground with purified quartz sand and the appropriate extracting reagent, for the determination of chlorophyll, Morgan's-soluble nutrients or "soluble iron."

Final yields were recorded on 30/9/48.

Results.

The results presented here represent a summary of the main data to be presented elsewhere later.

Visual observations.

Iron deficiency chlorosis was confined to the following treatments: $\text{Fe}_1\text{K}_1\text{C}_1$, $\text{Fe}_1\text{K}_2\text{C}_1$ (Fig. 1), $\text{Fe}_2\text{K}_1\text{C}_1$, $\text{Fe}_2\text{K}_2\text{C}_1$, $\text{Fe}_1\text{K}_1\text{C}_2$, $\text{Fe}_1\text{K}_2\text{C}_2$, $\text{Fe}_1\text{K}_3\text{C}_2$.

$\text{Fe}_1\text{K}_4\text{C}_2$, $\text{Fe}_2\text{K}_1\text{C}_2$, $\text{Fe}_2\text{K}_2\text{C}_2$, $\text{Fe}_2\text{K}_3\text{C}_2$, $\text{Fe}_3\text{K}_1\text{C}_2$ and $\text{Fe}_3\text{K}_2\text{C}_2$. A larger number of treatments therefore showed chlorosis in the C_2 than in the C_1 series. The chlorosis first seen in the lower iron treatments a month after planting became more severe as the season progressed. Chlorosis seen in the C_1 and the C_2 series was similar but where calcium carbonate had been added the chlorosis was more severe, and older leaves were also chlorotic.

Potassium deficiency symptoms appeared in the mid-stem region in all treatments at the K_1 and K_2 levels, and in the K_3C_2 and K_4C_2 treatments at the first two iron levels. Generally the symptoms first appeared in the low iron treatments and were more severe in the C_2 than in the corresponding C_1 series. Discolouration induced by potassium deficiency became especially marked in the higher iron treatments (Fe_3 and Fe_4) at the K_1 and K_2 levels. The symptoms spread rapidly to the apex of the plant and gave rise to a distinct yellow chlorosis which was accompanied by malformation and shrivelling of the lamina. The effect of iron in delaying the inception of the potassium deficiency symptoms and increasing their ultimate severity (Plate V, Fig. 2) previously reported (4) was thus confirmed.

Plants that showed both iron and potassium deficiency symptoms also developed "necrotic veining" in the young leaves (Fig. 2). These were usually pale green or yellow with a deep purple pigment in the veins, this was followed by the occurrence of irregular pale brown or buff necrotic areas on the lamina and of dark brown stem or petiole lesions in the young shoot. All treatments at the first iron level developed these symptoms: at the second iron level they occurred in all K treatments in the C_2 series, but only in the K_1 and K_2 treatments in the C_1 series.

It was shown previously (4) that plants starved of both iron and potassium became apically chlorotic but with abundant potassium at the same iron level the young leaves were green whereas the old leaves were distinctly paler. This "Fe K visual response" was confirmed here (Plate V, Fig. 1). The contrast diminished as the season advanced and was only seen at higher iron levels. A similar effect was noted in the C_2 series, but as iron deficiency chlorosis was more acute, the effect was confined to Fe_3 and Fe_4 treatments.

Two other effects observed were a forward rolling of the leaf margins in the mid-stem area seen in treatments Fe_3K_3 , Fe_3K_4 , Fe_4K_3 , Fe_4K_4 three months after planting, and an inward cupping, basal chlorosis and attenuation of the young leaves seen in Fe_3 and Fe_4 treatments at K_2 , K_3 and K_4 levels. These symptoms increased in severity with either iron or potassium level but were less severe with calcium carbonate.

Thus calcium carbonate did not alter the type of visual response observed in this experiment but accentuated iron and potassium deficiencies and necrotic veining or diminished symptoms suggestive of calcium deficiency.

Yield Data.

A part of the yield data are presented as mean values in Table 1.

The total dry weight of shoots increased with iron from Fe_1 to Fe_2 but thereafter declined (sharply in the C_1 , but more gradually in the C_2 series). Increased potassium supply increased the yield. The response to potassium was relatively greater, in the C_2 than in the C_1 series. Calcium carbonate reduced the yield; this reduction was relatively greater at the lower potassium levels.

The total dry weight of tubers (Table 1) increased with iron up to Fe_3 and then declined. The response to iron was relatively greater at the lower



Fig. 1. Effect of potassium on iron deficiency ($\text{Fe}_1\text{K}_1\text{C}_1$, $\text{Fe}_1\text{K}_2\text{C}_1$, $\text{Fe}_1\text{K}_3\text{C}_1$, $\text{Fe}_1\text{K}_4\text{C}_1$). K_1 and K_2 plants apically chlorotic; K_4 plant dark green at tip, paler in mid-stem area; K_3 intermediate. Note increased vigour with increased potassium supply.



Fig. 2. Effect of increased iron on potassium deficiency ($\text{Fe}_1\text{K}_1\text{C}_1$, $\text{Fe}_2\text{K}_1\text{C}_1$, $\text{Fe}_3\text{K}_1\text{C}_1$, $\text{Fe}_4\text{K}_1\text{C}_1$). Young leaves (top series): Marked increase in green colour with iron supply; appearance of marginal necrotic scorch in Fe_4 and slightly so in Fe_3 . Note absence of potassium deficiency in Fe_2 and marked chlorosis with necrotic veining in Fe_1 .

Old Leaves (bottom series): Note increased severity of potassium deficiency marginal scorch with iron supply and reduction of leaf size.

potassium levels and in the C_2 series. The response to potassium was relatively greater at the Fe_1 and Fe_2 levels and in the C_1 series. Calcium carbonate reduced the tuber yield.

TABLE I.
TOTAL DRY WEIGHT OF SHOOTS AND TUBERS (GMS./PLOT).

Treatment Level	Shoots		Tubers		Treatment Level	Shoots		Tubers	
	C_1	C_2	C_1	C_2		C_1	C_2	C_1	C_2
K_1	100.6	75.4	89.9	91.1	Fe_1	165.8	108.0	292.1	76.4
K_2	138.5	93.4	224.5	199.0	Fe_2	175.9	124.0	331.9	133.8
	(± 7.61)		(± 21.05)			(± 7.61)		(± 21.05)	
K_3	135.2	105.4	449.1	278.9	Fe_3	112.8	110.8	415.4	346.6
K_4	167.1	146.9	622.8	257.9	Fe_4	87.1	78.2	346.9	270.1

Chlorophyll.

For each month the chlorophyll content (Table II) in the young leaves (mean of all treatments) was higher than in the old leaves by 2-3 mg. g. dry weight. Mean chlorophyll content was greater in July and fell by 54.8% and 16.3% respectively, of the mean chlorophyll content of 6.36 mgms. per gm. of dry matter during August and September.

There was a strong positive response to iron in both young and old leaves (Table II). This response was greater in the young leaves. Increase of up to 93% of the mean content of the young leaves between Fe_1 and Fe_4 levels was associated with the K_1 and K_2 , rather than with the K_3 and K_4 treatments.

The application of potassium also increased (but less than iron) the chlorophyll content of both young and old leaves. The young leaves showed an increase of 20% from K_1 to K_3 and the old leaves 58% from K_1 to K_4 . It was noteworthy that the largest increase in chlorophyll content of the young leaves due to potassium was in the Fe_1 series, but in the old leaves the increase occurred equally at all levels of iron. The response to potassium was more pronounced for the C_1 than for the C_2 series for both young and old leaves.

Calcium carbonate decreased chlorophyll content for both young and old leaves and especially so in the low iron treatments.

The Dry Matter Content of the Lamina.

Numerical data are not given here. Old leaves had a higher % dry matter content than the young leaves for each month: the average difference was 15.8% of the mean. The highest dry matter content of the young leaves was in August and of the old leaves in August and September. Iron increased the dry matter in both young and old leaves, especially in the K_1 series. Potassium caused a marked decrease in the dry matter of old leaves and a similar but smaller decrease in young leaves of the C_2 series. Calcium carbonate reduced slightly the dry matter of both young and old leaves.

TABLE II.
CHLOROPHYLL CONTENT EXPRESSED AS MGMS. PER GM. OF DRY MATTER
—MEAN OF VALUES FOR MONTHS, JULY, AUGUST, SEPTEMBER, 1948.

		YOUNG LEAVES.						
		Fe ₁	Fe ₂	Fe ₃	Fe ₄	C ₁	C ₂	Mean
		(±1.382)				(±0.977)		(±0.691)
K ₁	..	2.14	3.76	8.89	12.02	7.70	5.70	6.70
K ₂	..	3.49	5.27	11.18	11.78	9.15	6.72	7.93
K ₃	..	5.41	5.99	11.65	9.98	10.00	6.51	8.25
K ₄	..	4.29	6.04	9.15	9.69	8.12	6.46	7.29
		(±0.977)				(±0.488)		
C ₁	..	5.37	7.42	10.76	11.42			8.74
C ₂	..	2.29	3.12	9.67	10.31			6.35
		(±0.691)				(±0.488)		
Mean	..	3.83	5.27	10.22	10.87	8.74	6.35	7.55
		OLD LEAVES.						
		(±1.304)				(±0.923)		(±0.653)
K ₁	..	2.55	2.47	3.27	4.90	3.63	2.97	3.30
K ₂	..	3.11	3.90	7.10	6.83	6.14	4.33	5.23
K ₃	..	4.78	4.12	7.20	7.50	7.50	4.30	5.90
K ₄	..	4.40	5.10	7.76	7.57	7.79	4.63	6.21
		(±0.923)				(±0.461)		
C ₁	..	5.17	5.29	7.67	6.98			6.26
C ₂	..	2.26	2.51	5.04	6.42			4.06
		(±0.653)				(±0.461)		
Mean	..	3.71	3.90	6.33	6.70	6.26	4.06	5.16

STANDARD ERRORS FOR (YOUNG - OLD) ARE :—

FeK Table	..	..	..	(±1.067)
CK and CFe Tables	..	..	..	(±0.755)
Border Means Fe, K	..	..	..	(±0.533)
Border Means C	..	..	..	(±0.378)

MEANS OF ALL VALUES FOR EACH MONTH.

	Young	Old	Mean
July	10.48	7.56	9.02
August	6.52	4.55	5.54
September	5.64	3.37	4.50
Mean	7.55	5.16	6.36

Soluble Iron and Other Nutrients.

Table III contains the soluble iron values obtained using 0.1 N hydrochloric acid saturated with ether or glycerophosphoric acid as extracting reagents and other elements extracted in Morgan's reagent.

The hydrochloric acid extracted more iron from the old than from the young leaves. Increased iron in the nutrient decreased the extractable iron content of the young leaves. Calcium carbonate had a similar effect for both young and old leaves, while increased potassium supply increased the "soluble" iron consistently in the old leaves and up to the K_3 level in the young. The soluble iron values obtained with glycerophosphoric acid mainly agreed with those obtained with hydrochloric acid with respect to effects of potassium supply and calcium carbonate, but less consistently with respect to iron supply.

An increased supply of iron decreased the extractable potassium in the young leaves at all potassium levels, but in the old leaves at the K_1 level only. Increased potassium supply increased the potassium extracted from both young and old leaves. Calcium carbonate increased the soluble potassium of the K_3 and K_4 treatments in the young leaves, but decreased it in old leaves at all K levels.

The old leaves yielded more soluble calcium than the young. Increased iron supply caused an overall decrease in soluble calcium of both young and old leaves. Increased potassium supply tended to decrease the soluble calcium extracted from the young leaves and also in the old leaves after the K_2 level. The application of calcium carbonate caused a distinct increase in both young and old leaves.

Increased iron supply decreased the soluble phosphorus in young leaves but had a variable effect in the old leaves. Increased potassium supply decreased the extractable phosphorus in both young and old leaves; this effect was relatively more pronounced in the absence of calcium carbonate in the young leaves. Calcium carbonate caused an overall increase in extractable phosphorus in the young leaves (due mainly to the K_3 and K_4 treatments) whereas there was a consistent decrease in the old leaves.

Ash Analysis Results.

The total iron, potassium, calcium and phosphorus contents of the laminae are presented in Table IV. The total iron content of the old leaves was always greater than that of the young. Increased iron supplies decreased iron in old but increased it in young leaves. Potassium had little effect on the total iron content of the young leaves but increased that in old leaves. Calcium carbonate caused a striking reduction in the iron content of the young leaves and a smaller one in the old leaves.

The total potassium content of the young leaves was greater than that of the old. Extra iron reduced the potassium content of young leaves, especially at the lower potassium levels. Increased potassium supplies increased the potassium content in both young and old leaves, especially in the Fe_3 and Fe_4 treatments.

The old leaves contained more total calcium than the young leaves. Increased supplies of iron increased the calcium content of the young leaves of the C_1 treatments and decreased it in old leaves. The calcium content increased with potassium supply up to the K_3 level (in young) or the K_2 level (in old leaves). Calcium carbonate increased the calcium content of both young and old leaves.

Young leaves contained more total phosphorus than old. Increased iron or potassium supply or the addition of calcium carbonate decreased the phosphorus content of both young and old leaves. The decrease in phosphorus content with iron supply was most marked in young leaves of the K_1 treatments.

TABLE IV.
TOTAL IRON, POTASSIUM, CALCIUM AND PHOSPHORUS CONTENTS; IRON EXPRESSED AS P.P.M., OTHERS AS % OF DRY MATTER.
Fe, K, Ca, P REPRESENT MEAN VALUES FOR THREE MONTHS.
YOUNG LEAVES.

Treatment Level	C ₁				C ₂				Treatment Level	C ₁				C ₂					
	Fe	K	Ca	P	Fe	K	Ca	P		Fe	K	Ca	P	Fe	K	Ca	P		
K ₁	215	2.51	1.71	0.89	146	2.04	2.39	0.77	Fe ₁	188	4.33	1.49	0.48	124	4.67	2.39	0.82		
K ₂	181	2.70	1.75	0.75	140	2.29	2.38	0.59		Fe ₂	181	3.29	1.68	0.70	123	3.89	2.62	0.66	
K ₃	225	3.13	1.87	0.58	135	3.26	2.41	0.56			Fe ₃	225	2.65	1.70	0.65	124	2.59	2.54	0.41
K ₄	220	4.54	1.40	0.58	142	6.24	2.39	0.50				Fe ₄	245	2.61	1.86	0.61	192	2.68	2.02
K ₁	OLD LEAVES								Fe ₁				309	1.69	3.72	0.59	281	1.93	7.16
	241	0.57	3.27	0.65	173	0.46	5.21	0.36		Fe ₂			281	1.53	3.55	0.52	260	1.86	7.31
	278	0.77	3.65	0.56	279	0.79	6.18	0.31			Fe ₃		273	2.14	3.02	0.49	254	2.00	5.96
	275	1.95	3.45	0.52	302	1.94	6.20	0.27				Fe ₄	260	2.21	3.09	0.49	243	2.68	3.03
K ₂	328	4.28	3.02	0.36	284	5.28	5.87	0.22											

The Inter-relationships of Chlorophyll, Soluble and Total Nutrients.

There are many relationships which can be deduced from the data in Tables 2, 3 and 4; some of the more important ones are noted here.

1. The description of the visual observations and the chlorophyll results (Table II) showed that potassium supply affected chlorophyll production. Although there was no direct correlation between potassium and chlorophyll content Fig. 3 shows that the chlorophyll content of the old leaves was related to the ratio of total (or soluble) potassium in young and old leaves. The sharp initial decline in this ratio was accompanied at first by a relatively small increase in chlorophyll content which thereafter increased rapidly. A similar trend was found for the chlorophyll in young leaves.
2. In the old leaves there was a distinct correlation between the soluble iron values (or to a lesser extent total iron) and either total or soluble potassium content (Fig. 4). The soluble iron content increased rapidly up to the 1% potassium value and gradually beyond this. No such correlation was obtained, however, in the young leaves.
3. It is seen from Fig. 5 that the chlorophyll content increased steadily with an increased total Fe/total P ratio in young leaves, but chlorophyll and total iron were not correlated.

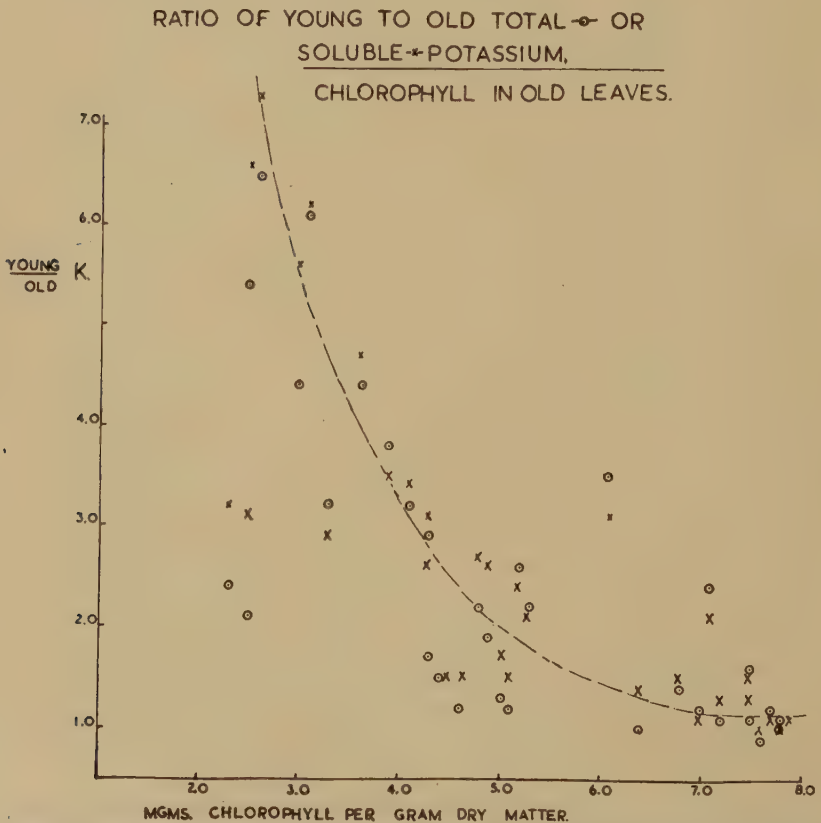


Fig. 3. Relationship between chlorophyll content of old leaves and the ratio values of young to old total or soluble potassium contents. Note that chlorophyll increased with a decrease in these values.

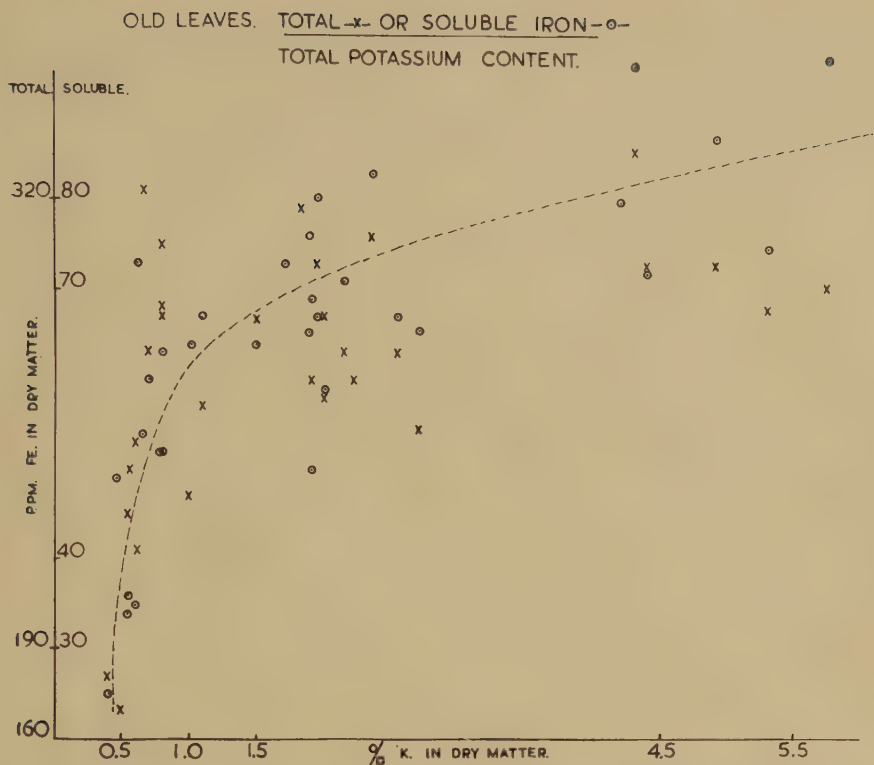


Fig. 4. The relationship between total or soluble iron content and potassium content of old leaves. Note sharp initial rise of iron content with increase in potassium content.

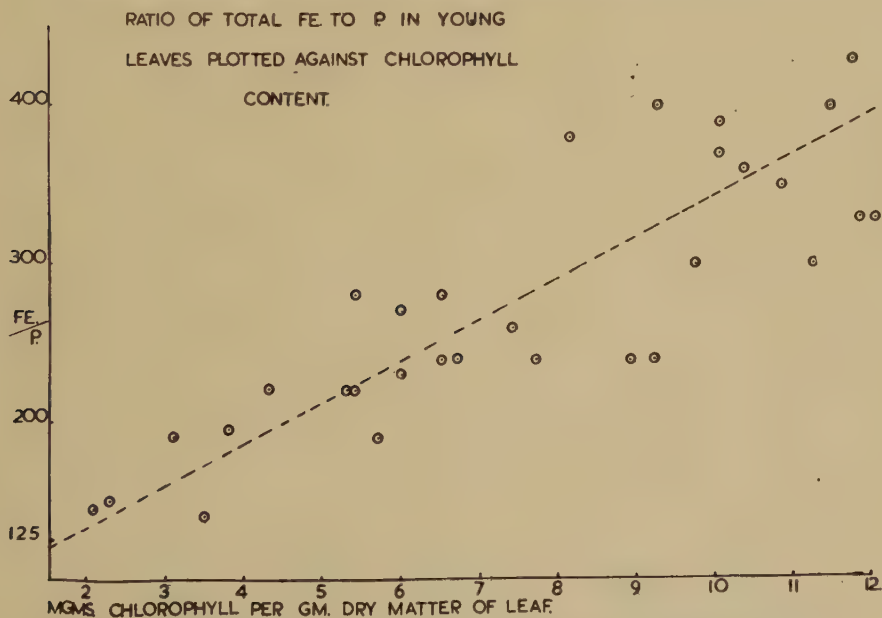


Fig. 5. The relationship between chlorophyll content of young leaves and the ratio values of total iron to total phosphorus content. Note steady increase of chlorophyll with increased Fe/P values.

4. Fig. 6 shows that chlorophyll increased as the ratio total Fe/total K content increased, especially in young leaves. At the low potassium levels relatively great increases in the Fe/K ratio were needed to influence chlorophyll content. At high potassium levels the effect of extra iron was minimised and chlorophyll formation was less dependent on the ratio.
5. As iron supply increased the proportion of total potassium retained in old leaves was increased.

Discussion.

Many of the effects previously described (4) were observed also in the presence of calcium carbonate. In particular were the change in the distribution of the chlorosis with added potassium, the delaying effect of iron on the inception of potassium deficiency and the decrease of the young/old leaf ratios of potassium content with increased iron supply.

The addition of calcium carbonate to the various treatments caused a decided increase in the pH of the sand which persisted until the end of the season. Its presence did not, however, cause any fundamental change in the symptoms or relationships observed, but merely modified the relative severity, critical concentration or time of appearance.

The yield data furnished evidence of the interaction of iron and potassium in the metabolism of the potato. The increases in tuber yield with iron or potassium were due to heavier tubers rather than to increase in numbers. Calcium carbonate, however, had the opposite effect as the decrease in tuber yield was accompanied by a marked increase in average weight at each level of iron and potassium.

The effect of potassium level on increased chlorophyll formation at low iron levels and the decreasing influence of potassium as the iron supply was increased suggested that iron was used more efficiently in the presence of adequate potassium. Further evidence in favour of this idea was furnished by (1) the steady decrease with extra potassium in the slope of the curve, (Fig. 6) relating Fe/K ratio (young leaves) to chlorophyll content; (2) the increase of both total and soluble iron content of the old leaves with increased potassium supply (Fig. 4); (3) the positive correlation of chlorophyll content with the young/old potassium leaf ratio (Fig. 3).

The apparent reversal of distribution of chlorosis which occurred when potassium was given to plants lacking both iron and potassium suggested that the distribution of the efficiency of iron utilisation was altered. Conversely the response to calcium carbonate implied that iron utilisation in the young leaves was still further impaired. These effects of potassium and calcium carbonate were reflected by the distribution of phosphorus between young and old leaves. Increased chlorophyll formation in the presence of extra iron was similarly associated with a reduced young to old leaf phosphorus ratio and in the young leaves increased chlorophyll content was correlated with an increased Fe/P ratio. These observations suggested that the rôle of potassium in mitigating, and of calcium carbonate in accentuating chlorosis might both be at least partially explained in terms of the redistribution of the phosphorus content with its consequent effect on iron metabolism.

One of the original intentions of this work was to test whether the effect of potassium on iron deficiency was related to the problem of lime induced chlorosis, in which a fairly consistent feature in the literature has been the presence of a high K/Ca ratio in chlorotic leaves (5) (6) (7) (8). The values

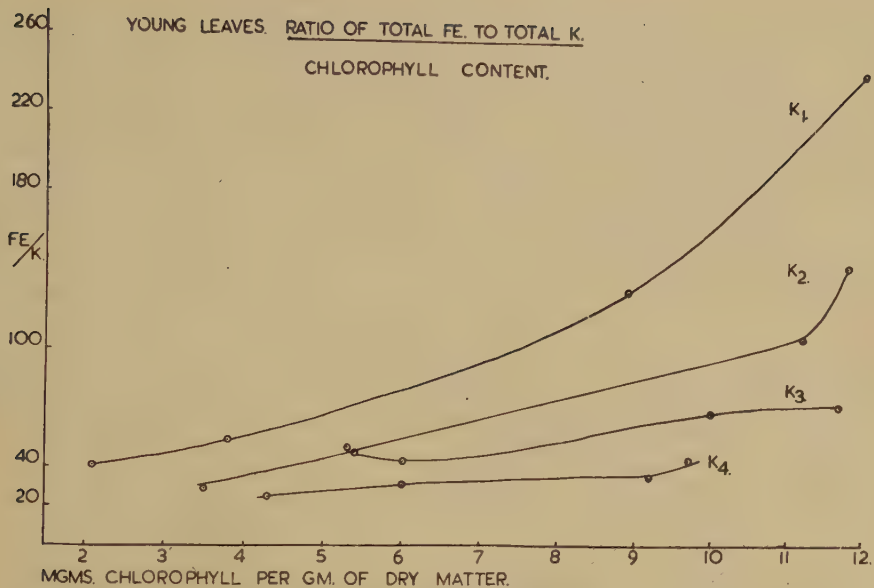


Fig. 6. Graphs showing relationship between chlorophyll content of young leaves and ratio values of total iron to total potassium content. Note that as potassium level was increased the variation in chlorophyll content became less dependent on Fe/K ratio.

of the K/Ca ratio in young and old leaves have been calculated. The results indicate a complex inter-relationship and data are not included here. Increase of iron supply generally reduced the K/Ca ratio in the young leaves (where chlorosis was most obvious) but increased it in the old leaves. Increased potassium from K₁ to K₃ also reduced the ratio for the Fe₁ treatments, but luxury levels of potassium caused a high K/Ca ratio and extra potassium also increased it when iron was provided at the Fe₂, Fe₃ and Fe₄ levels. Thus it was not possible to say that a high K/Ca ratio was mainly associated with chlorosis in this experiment, as opposed to conditions of lime-induced chlorosis in the field, since in the sand cultures calcium carbonate caused a lower K/Ca ratio in both young and old leaves. The apparent greater translocation of potassium from old to young leaves when lacking iron would, however, be conducive to an increase in the K/Ca ratio in young leaves of chlorotic plants as was found in this work and equally to a reduced K/Ca ratio in old leaves.

The data discussed indicate in a striking manner the point that whereas little correlation exists between the *absolute* concentration of any element in young or old leaves and chlorophyll content, certain marked consistent trends emerge when particular ratios are considered, e.g., between young and old leaves or Fe/P and Fe/K ratios. The reasons for these correlations are not understood at present. Young leaves often showed different inter-relationships from the old leaves. It is important, therefore, to discriminate between the two in work of this nature.

Summary.

1. Potato plants (variety, Majestic) were grown in sand culture with four levels of iron and four levels of potassium, with or without the addition of calcium carbonate to the sand, to produce severe

deficiency, mild deficiency, normal and luxury consumption effects of both iron and potassium.

2. The experiment confirmed the visual observations made previously. Additional symptoms included necrotic veining in plants deficient in both iron and potassium, forward rolling of leaves in plants given high iron and potassium together with suspected calcium deficiency.
3. Calcium carbonate did not alter the type of visual response observed but modified their severity, or the threshold levels at which they appeared.
4. Potassium or iron (up to the Fe_2 level) increased, whereas calcium carbonate decreased the total dry weight of shoots. Both total yield and average weight of tuber increased with iron up to the Fe_3 level and then decreased; calcium carbonate decreased the total yield, but increased the average weight, of tubers, potassium increased both.
5. Increased iron or potassium supply increased, and calcium carbonate decreased the chlorophyll content of young and old laminae. Young leaves contained more chlorophyll than old (dry weight basis). The concentration in both decreased rapidly as the season progressed.
6. Increased potassium supply increased, while calcium carbonate decreased soluble iron in old leaves (extracted by 0.1 N HCl and ether, or glycerophosphoric acid).
7. Increased iron supply decreased soluble potassium in young leaves and increased it in old leaves.
8. Increased potassium supply increased soluble and total potassium and decreased the phosphorus in old and young leaves. Total iron was increased in old leaves and calcium was usually reduced.
9. Calcium carbonate increased soluble and total potassium and calcium, and soluble phosphorus in young leaves and decreased the total iron and phosphorus in old and young leaves.
10. Increased iron decreased the ratio of potassium in young to old leaves. Chlorophyll increased in old leaves as the young to old leaf ratio of total or soluble potassium increased. Chlorophyll in young leaves increased as the ratios of total iron to phosphorus or potassium increased. The soluble iron content of the old leaves increased in proportion to the total potassium content of the lamina.

Acknowledgments.

This experiment comprised part of the plant nutrition programme maintained with the aid of special grants by the Agricultural Research Council to whom grateful acknowledgment is made. Special thanks are due to Dr. E. J. Hewitt for his advice and guidance during the work and to Francis K. Balfour, Esq., Blairgowrie, Perth, for his generous gift of the selected seed tubers used in this experiment. Messrs. B. A. Notton and P. A. Thompson ably assisted in the analytical and greenhouse work respectively and the photographic records were kindly made by Mr. G. H. Jones, A.R.P.S.

The writer is indebted to the Rothamsted Statistical Service for assistance in the lay-out and statistical analysis of the experiment.

REFERENCES.

- (1) COMAR, C. L., BENNE, E. J. and BUTEYN, E. K., 1943. Calibration of a photo-electric colorimeter for the determination of chlorophyll. *Ind. Eng. Chem. (Anal. Ed.)*, **15**, 524.
- (2) COMAR, C. L. and ZSCHEILE, F. P., 1942. Analysis of plant extracts for chlorophylls *a* and *b* by a photo-electric spectrophotometric method. *Plant Phys.*, **17**, 198.

- (3) HEWITT, E. J., 1947. A technique for large scale pot sand cultures. *Ann. Rept. Long Ashton Res. Sta.*, 1946, p. 37.
- (4) JONES, E. W. and HEWITT, E. J., 1950. Experiments on iron metabolism in plants. II. The inter-relationship of iron and potassium in the metabolism of the potato plant. *Ann. Rept. Long Ashton Res. Sta.*, 1949, p. 49.
- (5) LINDNER, R. C. and HARLEY, C. P., 1944. Nutrient inter-relations in lime induced chlorosis. *Plant Phys.*, **19**, 420.
- (6) THORNE, D. W. and WALLACE, A., 1944. Some factors affecting chlorosis of high lime soils I. Ferrous and Ferric iron. *Soil Sci.*, **57**, 299.
- (7) WALLACE, T., 1928. Investigations on chlorosis of fruit trees. II. The composition of leaves, bark and wood of current season's shoots in cases of lime-induced chlorosis. *J. Pomol.*, **7**, 172.
- (8) WALLACE, T. and MANN, C. E. T., 1926. Investigations on chlorosis of fruit trees. *J. Pomol.*, **5**, 115.

THE EFFECT OF MOLYBDENUM AND NITRATE STATUS ON YIELD AND ASCORBIC ACID CONTENT OF CAULIFLOWER PLANTS IN SAND CULTURE.

By S. C. AGARWALA.

Among the elements known to be essential for plant growth, molybdenum is peculiar in that, although it is required in very minute quantities, relatively high concentrations can be tolerated (Arnon and Stout, 1939, Hewitt and Jones, 1949, 1950, Warington, 1946). Molybdenum deficiency is known to cause among other effects, accumulation of nitrate and reduction of ascorbic acid concentration (Hewitt, Agarwala and Jones, 1950).

It seemed of interest to study the optimum requirements and range of tolerance of molybdenum by plants grown with high and low nitrate supply and the changes occurring in plant metabolism associated with increasing amounts of the element. Cauliflower plants were selected for the work as it was also desired to determine the precise conditions needed to reproduce, in sand culture, typical Whiptail symptoms attributed to molybdenum deficiency by Davies (1945), Hewitt and Jones (1947-50), Waring *et al.* (1947, 1948), Jones *et al.* (1950), Plant (1950), Jones and Dermott (1950).

The present paper summarises the results for yields and ascorbic and dehydroascorbic acid concentrations resulting from the different experimental treatments.

Material and Methods.

The methods of purification of sand and nutrient solutions, and of cleaning the pyrex containers (2 gallons capacity) were the same as used by Hewitt and Jones (1950).

Cauliflower seeds, variety Majestic, were sown directly in the purified leached sand with the help of a clean glass rod. Two levels of nitrate nitrogen, 6 and 24 mg. equivalents per litre were used. For each level of nitrate nitrogen, 8 concentrations of molybdenum (as sodium molybdate) between 0.000003 mg. equivalent and 1.2 mg. equivalents per litre were used.

The nitrate levels were adjusted by appropriate amounts of calcium nitrate, sodium nitrate and calcium sulphate. No attempt was made to compensate the high sulphate in the low nitrogen series or the additional sodium in the high nitrogen series. The calcium sulphate was prepared by precipitation from purified calcium chloride and sodium sulphate. Glass distilled water and purified nutrients were used for all levels of molybdenum and solutions were applied daily.

The composition of the nutrient solutions as mg. equivalents per litre was as follows :—

	6 mg.eq./litre NO ₃	24 mg.eq./litre NO ₃
	mg.eq./litre	mg.eq./litre
KNO ₃	4	4
Ca (NO ₃) ₂	2	10
CaSO ₄	8	—
MgSO ₄	2.25	2.25
NaNO ₃	—	10
NaH ₂ PO ₄	4 (as PO ₄ "')	4 (as PO ₄ "')
Fe as citrate	0.3	0.3
Cu, Zn	0.002	0.002
B''' as Boric acid	0.15	0.15
Co and Ni as sulphate	0.0002	0.0002
Ga	0.0002	0.0002

As the purified sand and glass distilled water supplied approximately 0.00003 mg. equivalents/litre of molybdenum no extra sodium molybdate was added for the lowest molybdenum level designated "nil" in the figures. The other levels of molybdenum used were 0.00003, 0.0003, .003, .03, .3, 3 and 1.2 mg. eq./litre corresponding to 0.00005, 0.0005, 0.005, 0.048, 0.48, 4.8 and 19.2 p.p.m. (nominal).

There were 4 replicates for each treatment, arranged in 2 randomised blocks. In each block there were 2 pots for each treatment.

Ascorbic acid (A.A.) was determined by rapid titration of the plant extract in 5% metaphosphoric acid with 2:6 dichloro-phenol-indophenol. In later determinations the tissues were macerated with 5% metaphosphoric acid containing 1% amidosulphonic acid to destroy any nitrite present which is likely to cause loss of ascorbic acid.

Dehydroascorbic acid (DHA) was determined by difference from increase in A.A. after reduction of D.H.A. by H₂S at pH 3-4. Excess of H₂S was removed by degassing with N₂ for 30 minutes.

The titre due to cysteine was determined by Mapson's (1943) method. The values of A.A. given after 55 days were corrected to exclude cysteine; earlier data include cysteine as ascorbic acid. No dihydroxymaleic acid was found.

Results.

Visual Effects.

Visual observations are described briefly to correlate them with the yield and ascorbic acid concentrations.

Molybdenum deficiency symptoms were observed in plants up to a level of 0.0005 p.p.m. in the high nitrate series and up to 0.00005 p.p.m. in the low nitrate series. At the lowest level of molybdenum typical symptoms of acute deficiency as described by Hewitt and Jones (1947, 1949, 1950) occurred. At 0.00005 p.p.m. Mo in the high nitrate series typical Whiptail symptoms as recorded in the field by Waring *et al.* (1947) and, Plant (1950 a b) developed (Fig. 1a).

In the low nitrate series the Whiptail effect was also observed but growth was much less and plants were not typical of those commonly found under field conditions. These effects and the essential conditions have been described briefly by Hewitt and Agarwala (1951).



Fig. 1a. Cauliflower plants (84 days old) grown with 0.00005 p.p.m. molybdenum and 24 mg.eq./litre nitrate nitrogen. Note the fully developed leaves at the base, younger leaves with defective lamina, and the "cull" heads.



Fig. 1b. Cauliflower plant (140 days old) grown with 0.0005 p.p.m. molybdenum and 24 mg.eq./litre nitrate nitrogen. Note the deformed younger leaves. Most of the older leaves had been removed.

The important features at the 0.00005 p.p.m. Mo level in the high nitrate series comprised initially vigorous growth and dark green foliage followed by breakdown of leaf margins, loss of lamina, death of the growing point, elongated bare petioles and fully expanded old leaves. "Cull" heads surrounded by stunted leaves with brown dead tips were also produced. Most of these symptoms were observed in sand culture plants grown by Hewitt and Jones (1947, 1949, 1950) but the vigorous growth and dark green foliage were usually absent and incidence of the symptoms could not be predicted for certain.

At 0.0005 p.p.m. slight deformation of lamina of younger leaves was observed in the high nitrate series at later stages of growth Plate VI (Fig. 1b). This was preceded by slight mottling of intervenal areas.

In general the growth and vigour of plants in the low nitrate series was markedly less than in the high nitrate series but growth appeared to be improved in the latter up to the maximum molybdenum levels.

A blue pigment, probably the same as reported by Warrington (1946), was found in the lower leaf epidermis and stem cortex of plants receiving 4.8 and 19.2 p.p.m. molybdenum in the low nitrate series and 19.2 p.p.m. molybdenum in the high nitrate series. At 38.4, and 76.8 p.p.m. molybdenum in both high and low nitrate series the blue pigmentation of the undersurface of the leaf was very marked. Growth and vigour seemed to be depressed only at the last two levels in a separate experiment using molybdenum concentrations between 9.6 and 76.8 p.p.m.

TABLE I.
EFFECT OF MOLYBDENUM CONCENTRATION AND
NITRATE SUPPLY ON THE YIELD OF CAULIFLOWERS IN SAND CULTURE.
(MEAN VALUES).

Conc. of molybdenum supplied p.p.m.	6 mg. eq./litre nitrogen. yield Dry wt. in gm.		24 mg. eq./litre nitrogen. yield Dry wt. in gm.	
	Total per plant	Curd per plant	Total per plant	Curd per plant
0.000005	3.96	0.0	8.15	0.0
0.00005	18.12	0.0	94.55	5.95
0.0005	76.15	3.27	157.90	13.30
0.005	68.25	4.35	213.85	18.35
0.048	74.50	3.82	223.55	41.82
0.48	79.50	3.40	224.85	48.70
4.8	64.10	3.10	270.05	51.80
19.2	67.80	3.61	270.60	58.0

SEPARATE EXPERIMENT.

Conc. of molybdenum supplied p.p.m.	9.5 mg. eq./litre nitrogen	29.5 mg. eq./litre nitrogen
	Total Yield gm.	Total Yield gm.
9.6	9.8	21.2
19.2	8.9	26.2
38.4	6.1	15.5
76.8	6.7	13.2

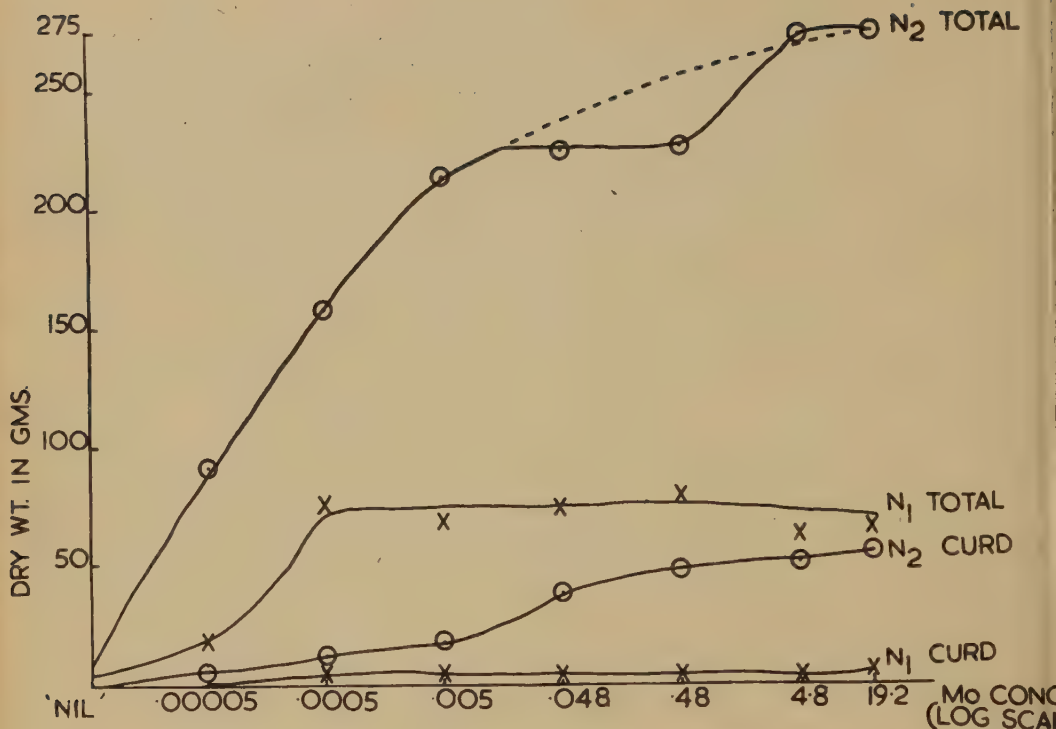


Fig. 2. Relation of molybdenum and nitrate concentration to total yield and yield of curd (as dry weight) of cauliflower plants grown in sand culture.

Yield Data.

Only the final yield values of plants grown for the full duration of the experiment are given in this paper in Table I and Figure 2.

It is clear that at all levels of molybdenum, including the lowest, the yields of plants receiving low nitrate nitrogen were less than the ones receiving high nitrate. Increase in molybdenum up to a level of 0.0005 p.p.m. resulted in greater yield under both high and low nitrate supply. Further increase in molybdenum showed a positive but decreasing effect on the yield in plants receiving high nitrate and none at all under low nitrate supply.

In a separate experiment the yield was depressed under both high and low nitrate supply by molybdenum levels of 38.4 and 76.8 p.p.m.

There was no curd formation at .000005 (nil) p.p.m. Mo. At .00005 p.p.m. small curds were produced with high nitrate supply in a few plants in which the growing point was not killed, but under low nitrate supply no curd was formed. Even at higher levels of molybdenum curd yields under low nitrate supply were very poor and bore little relation to molybdenum level. With high nitrate supply the curds were poor up to .005 p.p.m. molybdenum. Further increase in molybdenum increased the curd yields considerably up to the maximum level.

Ascorbic Acid.

The concentration of ascorbic acid in all parts of the plants examined, including roots, stem and leaves, was least at the lowest level of molybdenum and increased up to 0.0005 p.p.m. molybdenum. Beyond this level it did not

show any consistent change (Table II and Figs. 3 and 4.) This effect became pronounced from the 29th day onwards. The response was least pronounced in the first set of estimations when leaf formation was just beginning and before molybdenum deficiency effects were evident. The concentration of

TABLE III.
ASCORBIC ACID IN MGS. PER 100 GMS. FRESH LEAF MATERIAL OF CAULIFLOWERS—
MAJESTIC VARIETY. SPECIMENS FROM ACID SOIL AT BROMHAM (WILTS.). LOWER
GREENSAND pH 5.5.

		16/8/50	27/9/50
Control	Slight Whiptail	137	129
Gypsum, 3 ton p.a. .. .	Severe Whiptail	130*	106*
Gypsum, plus sodium molybdate .. .	Trace Whiptail	177	147
Ground limestone, 3 tons p.a. .. .	—	190*	127
Ground limestone, 6 tons p.a. .. .	—	191*	131
Sodium molybdate, 2 lbs. p.a. .. .	—	208*	145
Sodium molybdate, 4 lbs. p.a. .. .	—	194*	145
Sodium molybdate to seed bed .. .	—	176	138
		±5.41	±7.0

*Significantly less or greater than control treatments.

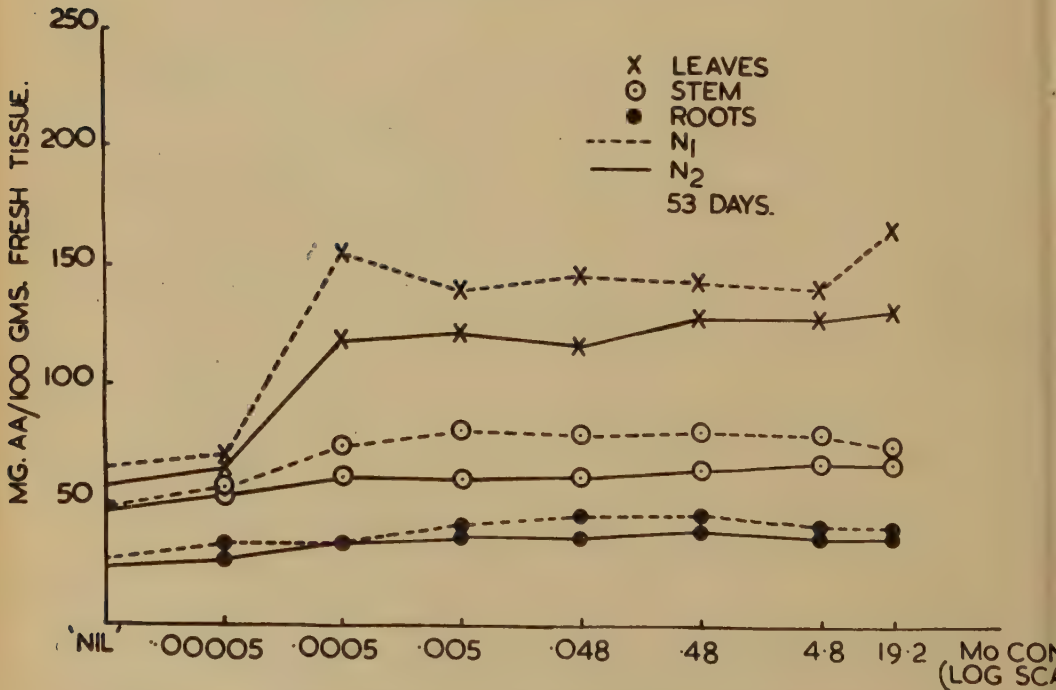


Fig. 3. Relation of molybdenum and nitrate concentration to ascorbic acid content in different parts of cauliflower plants after 53 days in sand culture.

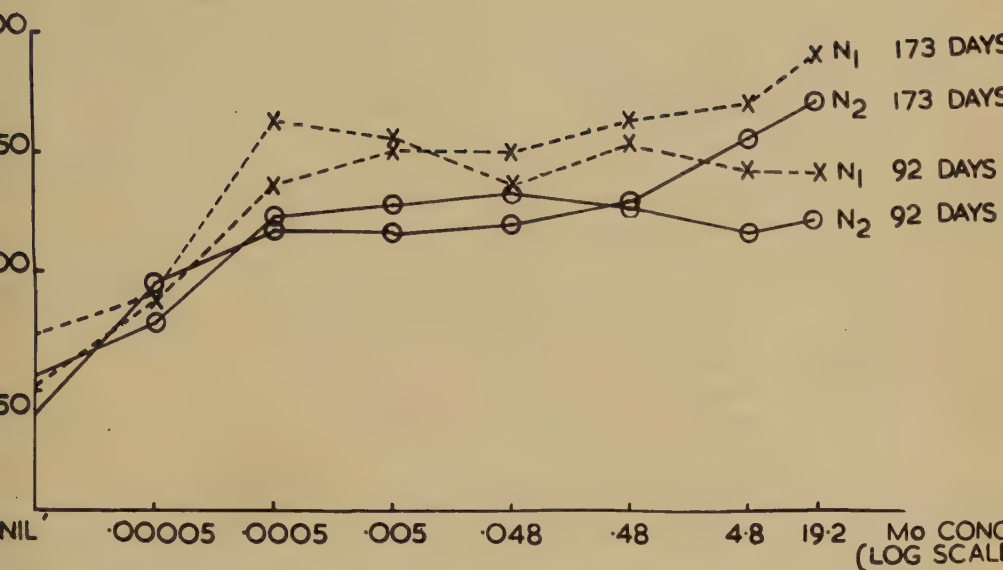


Fig. 4. Relation of molybdenum and nitrate concentration to ascorbic acid content in leaves of cauliflower plants at different stages of growth in sand culture.

ascorbic acid was highest in leaves and least in roots. No consistent difference was found between old and young leaves, although in most cases the A.A. was found to be more in younger leaves than the old.

The concentration of dehydroascorbic acid was variable and did not appear to bear any relation to the molybdenum or nitrate level.

The total ascorbic plus dehydroascorbic acid showed the same trend of results as ascorbic acid.

It appears that with one or two exceptions the concentration of ascorbic acid in plants receiving the higher nitrate treatment was less than those receiving lower nitrate.

Fractions other than ascorbic acid, e.g., cysteine, accounted for only 5% (rarely 10%) of the ascorbic acid values and their inclusion in the estimations up to 55th day does not appear to have affected the general conditions. Subsequent determinations included titration in the presence of formaldehyde and were corrected for the cysteine reductone fractions.

Field samples, kindly supplied by Dr. W. Plant from experimental plots on an acid soil, confirmed that the A.A. content (2:6 dichloro-indophenol titre) of plants showing molybdenum deficiency (control and Gypsum treated plots) was significantly lower than that of others given lime or molybdenum and growing normally.

Discussion.

The yield data show the importance of molybdenum in the growth and curd formation in cauliflower plants.

Growth was increased by molybdenum up to 0.0005 p.p.m. when limited nitrate was given but the effect of molybdenum persisted up to much higher levels (5 p.p.m. or more) when a high concentration of nitrate was available. Unpublished data obtained by the writer showed that considerable nitrate

accumulation occurred at molybdenum levels up to 0.00005 in both the high and low nitrogen series, but at higher molybdenum levels in the low nitrate series there was negligible nitrate whereas it persisted in the higher nitrate series. These results were consistent with effects reported by Hewitt and Jones (1947, 1949), Mulder (1948), Stout and Meagher (1948).

It is clear from the results that the concentration of ascorbic acid was lowered not only in the leaves, as reported by Hewitt, Agarwala and Jones (1950), but also in other parts of the plant when the molybdenum supply was reduced below 0.0005 p.p.m. This effect was not vitiated by the presence of other reductones, e.g., cysteine. The lowering of ascorbic acid concentration could not be accounted for by a corresponding increase in dehydroascorbic acid in the molybdenum deficient plants. Ascorbic acid was also depressed by high nitrate status. This possibility was anticipated by Hewitt *et al.* (1950) but further discussion of the problems raised will be deferred for a later publication.

Acknowledgment.

This investigation was planned by Dr. E. J. Hewitt, to whom the author is indebted for suggestions and helpful criticism. Acknowledgment is also made to Miss J. Mores for technical assistance. The work was carried out at Long Ashton during a period of study leave from the University of Lucknow with the aid of a grant from the Government of the United Province (India).

REFERENCES.

- ARNON, D. I., and STOUT, P. R. (1939). *Plant Physiol.* **14**, 599.
 DAVIES, E. B. (1945). *Nature*, **156**, 392.
 HEWITT, E. J. and JONES, E. W. (1947). *J. Pomol. & Hort. Sci.*, **23**, 254.
 HEWITT, E. J. and JONES, E. W. (1950). *Ann. Rept. Long Ashton*, 1949, p. 58.
 HEWITT, E. J., AGARWALA, S. C. and JONES, E. W. (1950). *Nature*, **166**, 1119.
 HEWITT, E. J. and AGARWALA, S. C. (1951). *Nature*, **167**, 733.
 JONES, J. O. and DERMOTT, W. (1950). *Nature*, **165**, 248.
 MAPSON, L. W. (1943). *J. Soc. Chem. Ind.*, **62**, 223.
 MULDER, E. G. (1948). *Plant and Soil*, **1**, 94.
 PLANT, W. (1950). *Nature*, **165**, 533.
 STOUT, P. R. and MEAGHER, W. R. (1948). *Science*, **108**, 471.
 WARINGTON, K. (1946). *Ann. appl. Biol.*, **23**, 249.
 WARING, E. J., SHIRLOW, N. S. and WILSON, R. D. (1947). *J. Aust. Inst. Agr. Sci.*, 187.
 WARING, E. J., WILSON, R. D. and SHIRLOW, N. S. (1948). *Agric. Gaz. N. S. Wales Miscell. Public.*, No. 3354.
 WILSON, R. D. and WARING, E. J. (1948). *J. Aust. Inst. Agri. Sci.*, **14**, 141.

By WILLIAM PLANT.

The molybdenum contents of the ground limestone and gypsum were found to be small.

	Water soluble Mo content p.p.m.	Total Mo supplied per acre (gm.)
Ground limestone. 3 tons p.a. ..	0.018	0.054
Gypsum. 3 tons p.a.	0.006	0.018

The calculated amount of molybdenum supplied in the limestone at the rate of 3 tons per acre would be incapable, according to previous calculations (6), of correcting the deficiency.

Cauliflower seeds (Var. Majestic) were sown 5/4/50. Planting out was done on 16/6/50 and harvesting took place during October.

Visual symptoms.

The first symptoms of molybdenum deficiency were noticed in the seed bed (25/5/50) and consisted of chlorosis, cupping and elongation of the leaves. The same syndrome has been described by Hewitt (1). The young plants showed a recovery before being planted out but subsequently the symptoms occurred again in the gypsum and control treatments. By the end of August the younger chlorotic leaves had either shrivelled up, fallen off or turned green, and the new leaves of the whiptail plants had developed strap-like petioles as described previously (6). By harvest time whiptail plants exhibited a wide range of symptoms consisting of blind or partially blind growing points, incomplete curds, some of them being interspersed with leaf-like bracts. In habit some plants were dwarf, some branched, and others had large spreading outside leaves with whiptail leaves placed centrally. Adventitious shoots underground were common. The plants in the remaining treatments were healthy.

Yields.

The cauliflower heads were harvested over a period of a month and graded at the time of picking into the following categories:—

- G. Giant : 4 heads per bushel box.
- L. Large : 6 heads per bushel box.
- M. Medium : 12 heads per bushel box.
- U. Ungraded (small heads).
- W. Whiptails.

The results are shown in Table I.

Apart from the gypsum treatment all others had a similar number of marketable heads in the different categories. The incidence of whiptail was highest on the gypsum only treatment (79%) followed by control (24%).

All treatments receiving lime or sodium molybdate were free from the deficiency apart from the presence of one or two whiptail plants on the gypsum + sodium molybdate treatment.

TABLE I.
THE PERCENTAGE OF MARKETABLE AND WHIPTAIL CAULIFLOWERS.

Treatment	Percentage Marketable Grades				Percentage Whiptail
	G	L	M	U	
Control	5	30	35	6	24
Gypsum. 3 tons p.a. ..	1	10	10	0	79
Gypsum + Sodium molybdate ..	8	28	41	19	4
Ground limestone. 3 tons p.a. ..	7	28	35	30	0
„ „ 6 tons p.a. ..	5	28	40	27	0
Sodium molybdate. 2 lb. p.a. ..	4	26	47	23	0
„ „ 4 lb. p.a. ..	0	32	45	23	0
Sodium molybdate to seed bed ..	5	30	39	26	0

It is interesting to note that the plants receiving molybdenum in the seed bed gave yields equal to those of plants supplied with molybdenum in the field.

Chemical data.

The contents of ascorbic acid, nitrate and molybdenum found in the leaves at two periods of sampling (16/8/50 and 27/9/50) are shown in Table II. Leaves were chosen from the plants at a position midway between the centre and outside. The petioles were removed and the lamina used for analysis. Molybdenum was estimated according to the method of Piper and Beckworth (1948); the nitrate method was adopted from Peech and English (1944); ascorbic acid was determined by rapid titration of leaf extracts in 5% metaphosphoric acid in copper-free water with 2-6 chlorophenol-indophenol.

Taking the figures from the molybdate treatments as representative of healthy growth, it can be seen that ascorbic acid is significantly lower and the nitrate higher in the gypsum treatment. This was more pronounced in the first sampling (16/8/50) when the leaves were chlorotic. In the control treatment where the deficiency was slight these two factors showed a similar tendency but to a smaller degree. It thus appears that the contents of ascorbic acid and nitrate tend to show a negative correlation at specific levels of molybdenum.

Although the molybdenum values are lowest and of the same order of magnitude on the Control, gypsum and sodium molybdate—seed bed treatments; the last failed to show deficiency symptoms. Whilst it appears that a molybdenum value below 0.10 p.p.m. may show the deficiency syndrome it does not invariably do so. The effect of ground limestone at 3 and 6 tons per acre gave an increase in molybdenum content in the plant comparable to the dressings of sodium molybdate. The molybdenum values of these treatments in the last sampling (27/9/50) were appreciably higher than the first, whilst the other treatments containing 0.10 p.p.m. molybdenum

TABLE II.
CONTENTS OF ASCORBIC ACID, NITRATE AND MOLYBDENUM IN CAULIFLOWER.
(VAR. MAJESTIC).

Treatment and date of sampling	Symptoms	Fresh wt.		Dry matter
		Asc. acid mg/100 gm.	NO ₃ , p.p.m.	Mo p.p.m.
16/8/50				
Control	slight whiptail (chlorosis)	173	1920	0.10
Gypsum. 3 tons p.a. . . .	severe whiptail (chlorosis)	130	4150	0.06
Gypsum + sodium molybdate ..	trace whiptail	177	1420	0.09
Ground limestone. 3 tons p.a. ..	none	190	1390	0.22
„ „ 6 tons p.a. ..	none	191	1310	0.39
Sodium molybdate. 2 lb. p.a. ..	none	208	1310	0.37
„ „ 4 lb. p.a. ..	none	194	1570	0.86
Sodium molybdate to seed bed ..	none	176	1510	0.09
Standard error		± 5.41	± 376.1	± 0.09
27/9/50.				
Control	slight whiptail (no chlorosis)	129	1880	0.08
Gypsum. 3 tons p.a. . . .	severe whiptail (no chlorosis)	106	2990	0.06
Gypsum + sodium molybdate ..	trace whiptail	147	2250	0.29
Ground limestone. 3 tons p.a. ..	none	127	1670	0.53
„ „ 6 tons p.a. ..	none	131	1580	0.81
Sodium molybdate. 2 lb. p.a. ..	none	145	1170	0.44
„ „ 4 lb. p.a. ..	none	145	1390	0.91
Sodium molybdate to seed bed ..	none	138	1500	0.08
Standard error		± 7.0	± 140.0	± 0.07

or less remained the same. It thus appears that if molybdenum is available for uptake by the roots, the amount in the plant will increase as development proceeds.

Summary.

- (1) An experiment with cauliflower was carried out on an acid Lower Greensand soil to determine the relationship between molybdenum deficiency and the contents of ascorbic acid, nitrate and molybdenum.
- (2) Treatments to vary the level of molybdenum consisted of applications of ground limestone, and varying combinations of sodium molybdate and gypsum, separately and together.

- (3) The syndrome of molybdenum deficiency which occurred on the control and gypsum treatments was characterised by the occurrence of chlorosis, malformation of lamina and blindness.
- (4) The incidence of whiptail was confined to the control (24%) and gypsum treatment (79%). At harvesting the cauliflower heads were graded. Ground limestone gave no better results than 2 lb. of sodium molybdate per acre.
- (5) Leaf analyses were carried out at two periods in the growing season to determine ascorbic acid, nitrate and molybdenum values.
- (6) Chlorotic leaves from whiptail plants contained significantly lower amounts of ascorbic acid and molybdenum and a significantly higher amount of nitrate. These correlations were less noticeable as chlorosis disappeared, but were still significant in older plants of typical whiptail appearance.

Acknowledgments.

The work was carried out under grants made by the Agricultural Research Council to whom grateful acknowledgment is made.

My thanks are particularly due to Mr. W. J. Redmond for carrying out the nitrate and molybdenum estimations and to Dr. S. C. Agarwala for determining the ascorbic acid values.

REFERENCES.

- (1) HEWITT, E. J. and JONES, E. W. (1947). The production of molybdenum deficiency in plants in sand culture with special reference to tomato and brassica crops. *J. Pomol.*, **23**, 254.
- (2) HEWITT, E. J., AGARWALA, S. C. and JONES, E. W. (1950). Effect of molybdenum status on the ascorbic acid content of plants in sand culture. *Nature*, **166**, 1119.
- (3) PEECH, M. and ENGLISH, L. (1944). Rapid micro-chemical soil tests. *Soil Science*, **57**, 167.
- (4) PIPER, C. S. and BECKWORTH, R. S. (1948). A new method for the determination of small amounts of molybdenum in plants. *J. Soc. Chem. Ind.*, **67**, 374.
- (5) PLANT, W. (1950). The use of lime and sodium molybdate for the control of "whiptail" in broccoli. *Nature*, **165**, 533.
- (6) PLANT, W. (1950). The control of "whiptail" in broccoli and cauliflower. *Agriculture*, **57**, 130-134.

SOME EFFECTS OF METALS IN EXCESS ON CROP PLANTS GROWN IN SOIL CULTURE.

I. EFFECTS OF COPPER, ZINC, LEAD, COBALT, NICKEL AND MANGANESE ON TOMATO GROWN IN AN ACID SOIL.

By D. J. D. NICHOLAS.

II. EFFECTS OF COPPER AND ZINC ON CROP PLANTS GROWN IN A VARIETY OF SOILS.

By W. A. FORSTER.

It is well known that metals present at high levels in the substrate can induce toxicity effects in crop plants. Thus the effects of excess Cu on plant growth have been studied by Willis and Piland (29), Chapman *et al.* (4), Liebig *et al.* (15), and Erkama (7). Somers and Shive (25) have shown that excess Mn in water culture results in iron deficiency symptoms in soya bean, and Hopkins *et al.* (13) and McGeorge (18) have confirmed this in pineapple grown in certain manganiferous soils in Puerto Rico and Hawaii respectively. In Britain, however, excess Mn in crops growing in strongly acid soils, does not appear to affect visibly the iron metabolism of the plants (21, 27).

In water culture experiments Brenchley (2) showed that excess Co or Ni resulted in iron deficiency symptoms and Somers and Shive (25) confirmed the effects of Co-induced chlorosis. Haselhoff (9) and Cotton (6) have noted chlorosis of younger leaves in plants given high levels of Ni. Millikan (19) has shown that an excess of Mn, Zn, Cu, Ni and Co respectively induced iron deficiency in flax grown in water culture and that the addition of Mo alleviated the chlorosis. Hewitt (10) on the other hand has demonstrated that additions of molybdenum aggravated the symptoms of iron deficiency induced by high levels of Cr^{+++} Mn^{++} Cu^{++} and Zn^{++} respectively in sugar beet grown in sand culture.

In Britain, instances of metal-induced iron deficiency are known in the field. Thus in parts of Somerset, Cornwall and North Wales, susceptible crops often show iron deficiency effects induced by metals present in soil adjacent to lead or tin mines. Moreover, when large amounts of industrial effluent are incorporated in soil, metal-induced iron deficiencies, in particular Zn and Cu, often appear in cereals, root and brassica crops (28).

In the first part of this investigation, the effects of Cu, Zn, Pb, Mn, Co and Ni respectively at 0, 5, 10 milli-equivalent (m.eq.) rates were studied in tomato grown in a factorial pot experiment in an acid soil (pH 5.5). A method was devised to extract the metals from fresh plant tissues in pyrex glass macerators. The metal content, and iron status of the homogenate was determined as well as the total chlorophyll content and chlorophylls A and B. It is shown that Co and Ni are more toxic, at similar m.eq. rates, than Cu, Pb, Zn or Mn. Manganese, although present in the plants at significantly higher levels than the other metals, did not result in symptoms of iron deficiency.

In Part II further work is reported on the effects of Zn and Cu in various soils on other crops including cauliflower, oat, and sugar beet grown in pot

culture. The injurious effects of the two metals were greater in acid soils and Cu was more toxic than Zn at similar m.eq. levels. Tomato was more susceptible to metal injury than sugar beet and cauliflower grown in the same soil.

I. EFFECTS OF COPPER, ZINC, LEAD, COBALT, NICKEL AND MANGANESE ON TOMATO GROWN IN ACID SOIL.

Experimental.

Pot Experiment.

The soil used was a sandy loam derived from the Ross series (pH 5.5). The following treatments were given in each of three blocks: Cu, Zn, Co, Ni, Pb and Mn respectively at 0, 5, and 10 m.eq. of metal. All were supplied as the sulphate except Pb which was given as the acetate. Each treatment, composed of 3, 10" pots treated with bitumen, was completely randomised.

The tomato seeds (var. Market King) were grown in sterilized John Innes compost. A fortnight later, the seedlings were transplanted into 162, 10" pots, one seedling per pot, and when they had become established, were given a supplementary nutrient solution of ammonium phosphate and potassium nitrate and magnesium sulphate as the soil was low in N, P, K and Mg. The metals were applied in solution (500 ml) every other day. When symptoms appeared, the application of metal was given once weekly, and was discontinued when symptoms were severe.

Laboratory Procedure

The plants were sampled twice during the growing season. The plant "tops" were divided into young leaves, old leaves and stem respectively. The following determinations were made: fresh weight of tops, total chlorophyll and the ratios of chlorophyll components A and B, metal content and iron status of young and old leaves. In addition, an attempt was made to fractionate the metals in fresh leaf tissue by extracting with glass distilled water in pyrex glass macerators. The metals were determined in the extracts and also in the residues. The determination of metal contents and iron levels in the ash of young, old leaves and stems respectively is in progress.

Maceration Method.

Fresh strips of young and old leaves respectively taken across the leaf blade, were extracted with glass distilled water in a special pyrex glass macerator. The macerator consists of a pyrex glass plunger which is ground at the base, fitting into a robust, hard glass tube. The plunger is rotated electrically at speed by means of a stirrer head. 2 gm of leaf tissue cut into small portions with a sharp stainless steel knife, and 2 ml of glass distilled water, are put into the macerator tube and the plunger rotated slowly at first, and faster later as the tissue is ground. The material is completely comminuted within a few minutes. The plunger is washed with 10 ml of glass distilled water and the supernatant and residue transferred into a 50 ml centrifuge tube which is immersed in a waterbath for 15 min. at 80° C. The material is centrifuged at 3,000 revs. per min. for 15 min.

Ion Exchange Method.

The supernatant solution is percolated through a 15 ml pyrex glass centrifuge tube packed with glass wool and provided with a hole at the base.

The glass wool is previously digested with re-distilled nitric acid and washed thoroughly with glass distilled water to eliminate traces of metal. The percolate free from suspended material is now passed through a similar tube packed with about 5 gm of cation exchange resin, Amberlite IR 100 or Zeocarb 215, so that the metals are exchanged, concentrated and separated from the anions. The elimination of the anions is, particularly useful as many of these interfere with the chemical tests for the metals, in particular phosphates.

The column is then eluted with 200 ml of 2N.HCl (re-distilled) delivered slowly from a pyrex burette. The cations are contained in the eluant. Although some of the metals can be determined direct in this extract, the Cl ions interfere with some of the tests, in particular Mn, and the eluant may be slightly coloured. The percolate is therefore evaporated to approximately 20 ml and 10 ml of nitric and perchloric acids added respectively and the digestion continued to the "incipient" dryness stage (21). About 20 ml of glass distilled water is added and the solution is boiled for 15 min., cooled and made up to 25 ml. Appropriate aliquots are taken for the chemical tests.

Even A.R. grade resins contain traces of Fe, Zn and Cu. These are removed by percolating a 5% solution of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ continuously through the resin in a special glass percolater (1) until the trace metals cannot be detected in the leachate. This may take 48 hrs. as Mg exchanges slowly with Fe, Cu and Zn in the resin bed. The MgSO_4 solution is removed and 2N.HCl (re-distilled) introduced into the system and percolation with acid, which is renewed at intervals, continued until no more Mg is detected in the eluant. The resin is then leached with glass distilled water to remove free H ions, until the pH becomes constant.

Chemical Methods.

The residues left after maceration were digested with nitric and perchloric acids and the metals determined therein.

Copper and zinc and lead were determined after separation of the three metals with dithizone in CCl_4 (12). Copper was determined as copper diethyldithiocarbamate in CCl_4 ; zinc as a dithizonate, a monocolour method (12), and Pb as a dithizonate, a mixed colour method (24). Iron was determined with α -dipyridyl (20), nickel with dimethylglyoxime (14-24), cobalt with nitroso-R-salt (24), and manganese with potassium periodate (21). The methods were adapted to detect a metal specifically in the presence of the others (22).

Total chlorophyll was determined in acetone extracts of fresh leaves and the percentage transmittance of ether extracts of chlorophyll was determined with a quartz spectrophotometer (5). Moreover the ratio of chlorophylls A to B was also determined in the plants.

Results.

Visual Symptoms.

Details of the symptoms in tomato induced by metal excesses are given in Appendix I. The first iron deficiency effects appeared in the young leaves of plants given Co or Ni at the 10 m.eq. rate, and later severe intervenal and stem necrosis occurred which resulted in death of the growing points. Cu or Zn dosages resulted in slight iron deficiency effects, whereas Pb and Mn at similar m.eq. levels did not visibly affect the iron metabolism.

Fresh Weights.

The statistical analysis of the yields of plant "tops" for the 1st sampling are given in Table I.

TABLE I.

1st Sampling, 4-13/7/50.

THE EFFECT OF METAL APPLICATION ON FRESH WEIGHTS OF TOMATO "TOPS" (oz.).

Rate of metal applied (m.eq. per pot)	0	5	10	L.S.D. P=0.01
Mean of 3 blocks ..	8.43	7.64	7.15	0.83
Order	1	2	3	i.e. 1 > 3

Interaction between metals and rates was not significant.

The fresh weight yields have been significantly depressed by metal application. The interaction between metals and rates was not significant at the first sampling, but at the second sampling was highly significant ($P=0.01$) as shown in Table II.

TABLE II.

2nd Sampling.

THE EFFECT OF METAL APPLICATION ON FRESH WEIGHTS OF TOMATO "TOPS" (oz.).

Rate of Metal applied per pot m.eq.	Cu	Zn	Pb	Co	Ni	Mn	L.S.D. P=0.01
0	21.83	23.25	18.42	20.83	23.83	23.25	N.S.
5	22.08	15.58	13.42	11.08	17.08	24.00	7.14
10	10.08	13.00	5.33	5.08	3.00	18.42	7.14

Interaction between metals and rates was significant at $P=0.01$.

Rates of Metal Application.

At 5 m.eq. $Mn > Zn, Pb, Co$.

„ 10 m.eq. $\begin{cases} Mn > Pb, Co, Ni, Cu. \\ Zn > Pb, Co, Ni. \end{cases}$

Metal.	Rates of application of metal m.eq.
Copper ..	0, 5 > 10.
Zinc	0 > 5 and 10.
Lead	0, 5 > 10.
Cobalt ..	0 > 5 and 10.
Nickel ..	0, 5 > 10.
Manganese ..	not significant.

The results show that Mn has not reduced the fresh weight of tomato tops. At 10 m.eq. rates all other metals have significantly reduced the yields by comparison with the controls.

Chemical Data.

Metal Content.

Macerater Extracts. The metal content of the macerater extracts, and residues of young and old leaves as μg per gm fresh weight, are given in Table III. There is a tendency for more Cu, Zn, Pb, Ni to be present in the residue than in the water extract, and vice versa for Co, whereas Mn levels are similar in both fractions. The Cu, Co and Mn contents of young and old leaves are similar. There is more Zn and Pb in the old leaves whereas Ni tends to accumulate in the young leaves.

The statistical analysis of the metal content of macerater extracts for the 1st sampling is given in Table IV. The values are converted to $\log_{10}$.

In both young and old leaves the interaction between metals and rates was highly significant ($P = 0.001$). In the absence of applied metal, Mn and Zn contents are significantly greater than Pb and Co in extracts of young or old leaves. Thus the acid soil contains more available Mn and Zn than other metals. The Mn status of young and old leaves are significantly greater than for other metals when applied at similar m.eq. rates. Despite the accumulation of Mn, the plants were not visibly affected with iron deficiency, whereas a much lower level of Co resulted in an iron chlorosis, and later direct metal injury, viz. necrosis of leaves and stems. Nickel also produced similar visual effects to Co, but Ni was not detected in water extracts of the leaves.

The statistical data also show that Mn and Co contents at 5 or 10 m.eq. are significantly higher than in the control plants, but no increase is apparent, for Pb or Zn in tissue extracts with increased rates of application of these metals.

The increase in metal applied has significantly decreased the iron content of the macerater extracts of the young leaves. A similar effect was produced in the old leaves but differences in this instance are significant at the 5% level only.

Residues left after Maceration. The statistical data for the metal content of the residues of young and old leaves respectively are given in Table VI.

At the three rates of application, Mn is significantly greater than any other metal in the residues of both young and old leaves, as in the extracts. Co and Ni levels are significantly less than those of Mn or Zn in the young and old leaves. Thus Co and Ni are more toxic at lower levels within the plants.

The application of Mn, Co, Ni, Zn, Pb respectively at 5 and 10 m.eq. rates has resulted in significantly greater amounts of these metals in the residues than in those of the control plants. The Cu status of the residues, however, does not reflect the rates of Cu application. It is noteworthy that the application of Zn or Ni is better shown by the "residues" than by water extracts of leaves.

The iron content of the "residues" of young or old leaves showed no significant differences for the metals or rates of application.

The metal content at the second sampling made three weeks later showed similar trends to those of the first sampling.

TABLE III.

*1st Sampling, 4-13/7/50.*RANGE OF VALUES FOR THE METALS, AS μg PER GM. FRESH WEIGHT, IN MACERATED EXTRACTS AND RESIDUES OF YOUNG AND OLD TOMATO LEAVES.

Metal	Rate m.eq. per pot	Young Leaf		Old Leaf	
		water extract	residue	water extract	residue
Cu	0	1.0 - 1.45	6.0- 8.5	0.5- 2.5	1.3 - 2.5
	5	1.3 - 1.9	6.0- 8.5	1.3- 2.0	4.0 - 10.0
	10	—	6.0- 9.2	—	4.0 - 11.5
Zn	0	12.5 - 23.0	35.5- 43.0	18.8- 39.2	28.0 - 50.0
	5	11.3 - 54.2	51.0- 52.0	13.8- 76.5	56.0 - 69.0
	10	18.8 - 32.5	61.0-127.0	18.8- 66.5	85.0 -108.0
Pb	0	1.9 - 6.3	6.2- 7.8	4.0- 6.5	5.3 - 7.0
	5	3.0 - 3.5	8.5- 12.5	4.0- 15.25	17.6 - 35.5
	10	2.5 - 4.2	10.5- 15.5	6.9- 12.0	23.5 - 56.5
Co	0	1.25- 3.8	2.0- 3.5	0.8- 1.3	1.25- 2.5
	5	13.1 - 48.1	4.0- 12.5	8.8- 20.0	4.0 - 10.0
	10	6.9 - 32.5	8.5- 20.0	16.9- 65.0	4.0 - 11.5
Ni	0	not detected	not detected	not detected	not detected
	5	„	7.0- 13.0	„	2.5 - 6.0
	10	„	13.5- 21.3	„	8.5 - 12.5
Mn	0	38.0 - 47.5	50.0- 80.0	47.5-118.0	55.0 -105.0
	5	90.0 -200.0	280.0-305.0	115.0-260.0	202.0 -227.0
	10	118.0 -146.0	195.0-440.0	115.0-203.0	175.0 -360.0

TABLE IV.

*1st Sampling, 4-13/7/50.*THE METAL CONTENT OF THE MACERATER EXTRACTS (GLASS DISTILLED WATER)
OF TOMATO LEAVES.Young leaf— $\log_{10} \mu\text{g}$ per gm. fresh weight.Old leaf— $\log_{10} \times 10$; μg per gm. fresh weight.

Rate of metal applied m.eq. per pot	Leaf, Young (Y) Old (O)	Co	Pb	Mn	Zn	L.S.D. P=0.001
0	Y	0.3563	0.5589	1.6195	1.3179	0.5943
	O	1.0230	1.6929	2.8516	2.4138	0.6507
5	Y	1.3711	0.5011	2.1408	1.4288	0.5943
	O	2.0617	1.8392	3.2671	2.6324	0.6507
10	Y	1.3801	0.5400	2.1295	1.4235	0.5943
	O	2.4720	1.9427	3.1908	2.4974	0.6507

Interaction between metals and rates significant at P=0.001.

Metal Rate in m.eq.—P=0.001.

- At 0 m.eq. Young Leaf—Mn and Zn > Pb and Co.
Old Leaf—Mn and Zn > Pb > Co.
- At 5 m.eq. Young Leaf—Mn > Zn, Co > Pb.
Old Leaf—Mn > Co and Pb; Zn > Pb.
- At 10 m.eq. Young Leaf—Mn > Zn, Co > Pb.
Old Leaf—Mn > Zn, Co, Pb.

Metals. Metal Rates m.eq.—P=0.001.

- Co Young Leaf 10, 5 > 0.
Old Leaf 10, 5 > 0.
- Pb Young Leaf—not significant.
Old Leaf—not significant.
- Mn Young Leaf 10, 5 > 0 } P=0.05 only.
Old Leaf 10, 5 > 0. }
- Zn Young Leaf—not significant.
Old Leaf—not significant.

The iron content of the extracts are given in Table V.

TABLE V.

1st Sampling.

THE EFFECT OF METAL APPLICATION
ON THE IRON CONTENT OF MACERATER EXTRACTS OF TOMATO LEAVES.
Values $\text{Log}_{10} \mu\text{g Fe per gm. fresh weight.}$

Rate of Metal applied m.eq. per pot	Leaf Young (Y) Old (O)	0	5	10	L.S.D.
$\text{Log}_{10} \text{Fe } \mu\text{g per gm. fresh weight}$	Y	0.6283	0.5406	0.4133	P=0.01 0.1613
$\text{Log}_{10} \text{Fe } \mu\text{g per gm. fresh weight}$	O	0.7270	0.5575	0.5355	P=0.05 0.1196

Interaction between metals and rates was not significant.
Young leaf—0 > 10 m.eq. metal applied.
Old Leaf—0 > 5 & 10 m.eq. metal applied.

TABLE VI.

1st Sampling, 4-13/7/50.

THE METAL CONTENT OF THE "RESIDUES" AFTER MACERATION OF TOMATO LEAVES.
 $\text{Log}_{10} \mu\text{g metal per gm. fresh weight.}$

Rate of metal applied m.eq. per pot	Leaf- Young (Y) Old (O)	Cu	Zn	Pb	Co	Ni	Mn	L.S.D. P=0.001
0	Y	0.8628	1.5897	0.8337	0.4143	0.2274	1.7670	0.3941
	O	0.8223	1.5734	0.7812	0.1972	0.0414	1.9053	0.3787
5	Y	0.8424	1.7104	0.9998	0.8373	0.9447	2.4656	0.3941
	O	0.9416	1.7884	1.4271	0.8257	0.6250	2.3292	0.3787
10	Y	0.8664	1.8984	1.1139	1.0768	1.1963	2.4495	0.3941
	O	1.0402	1.9723	1.5732	0.9810	1.0290	2.4393	0.3787

Interaction between metals and rates significant at P=0.001.

<i>Metal</i>	<i>Rate m.eq.—P=0.001.</i>
0	Young Leaf—Mn, Zn > Cu, Pb > Co, Ni. Old Leaf—Mn > Zn > Cu, Pb > Co, Ni.
5	Young Leaf—Mn > Zn > Pb, Ni, Cu, Co. Old Leaf—Mn > Zn, Pb > Ni, Cu, Co.
10	Young Leaf—Mn > Zn > Pb, Ni, Cu, Co. Old Leaf—Mn > Zn > Pb > Ni, Cu, Co.

<i>Metal</i>	<i>Rates m.eq.—P=0.001.</i>
Cu	Young Leaf—no significant difference. Old Leaf—no significant difference.
Zn	Young Leaf—10, 5 > 0. } $P=0.01.$ Old Leaf— 10, 5 > 0. }
Pb	Young Leaf—no significant difference. Old Leaf—10, 5 > 0.
Co	Young Leaf—10, 5 > 0. Old Leaf—10, 5 > 0.
Ni	Young Leaf—10, 5 > 0. Old Leaf—10 > 5 > 0.
Mn	Young Leaf—10, 5 > 0. Old Leaf—10, 5 > 0.

Ash Analysis.

The determination of the metals in the ash of stem, young and old leaves is in progress. The values for Pb, Co, Ni, and Mn respectively in the ash are given in Table VII together with statistical analysis of the data.

TABLE VII.

THE RANGE OF VALUES FOR Pb, Co, Ni AND Mn RESPECTIVELY FOUND IN THE ASH OF STEM, YOUNG LEAF AND OLD LEAF OF TOMATO TREATED WITH THESE METALS.

Metal	Rate applied m.eq. per pot	Stem	Young Leaf	Old Leaf
Pb	0	6.0-9.8	7.3-9.4	9.8-12.3
	5	40.1-118.0	21.4-40.2	79.2-140.9
	10	20.0-316.3	14.0-42.3	98.0-275.6
Co	0	7.2	Not detected	0.8-1.5
	5	50.4-102.0	96.0-156.0	96.3-237.0
	10	94.4-147.0	73.6	182.6-185.4
Ni	0	Not detected	Not detected	Not detected
	5	31.5-39.1	87.3-111.3	95.6-124.1
	10	48.6-88.9	148.9-223.8	180.7- 211.9
Mn	0	219.7-394.9	577.4-766.2	1392.1-1416.5
	5	1155.6-2285.1	1662.1-6361.8	3247.8-6519.4
	10	1271.0-1842.2	1980.8-4281.6	3337.9-5531.8

<i>Metal.</i>	<i>Rates of application.</i>	<i>Plant Portions (stem, young leaf and old leaf).</i>
Pb	0	No significant differences between levels of Pb in plant parts.
	5	
	10	
Co	0	No significant differences between levels of Co in plant parts.
	5	
	10	
Ni	0*	Not significant.
	5	Old leaf, young leaf > stem at 0.001 levels.
	10	Old leaf, young leaf > stem at 0.001 levels.
Mn	0	No significant differences between levels of Mn in plant parts.
	5	
	10	

*Interaction between rates and plant portions are significant for Ni ($P=0.001$).

<i>Metal.</i>	<i>Plant Portion.</i>	<i>Rates of metal applied as m.eq. per pot.</i>
Pb	stem*	10, 5 > 0 at 0.001 levels
	young leaf	10, 5 > 0 at 0.001 levels.
	old leaf	10, 5 > 0 at 0.001 levels.
Co	all portions	10, 5 > 0.
Ni	stem*	10 > 5 > 0 at 0.001 level.
	young leaf	10 > 5 > 0 at 0.001 level.
	old leaf	10 > 5 > 0 at 0.001 level.
Mn	all portions	10, 5 > 0 at 0.001 level.

The results in Table VII show that there are no significant differences between the distribution of either Pb or Co in the ash of leaves and stems of tomato. The leaves contain more Ni and Mn than the stems when these metals are supplied to the plants.

The metal applications are shown equally well by the ash content of the three plant portions except for Pb where young leaf is not a sensitive indicator of Pb treatment ($P = 0.05$). In this connection, Pb in macerate extracts and "residues" of young leaf did not reflect treatment so that this metal is probably immobilised in the stem and in particular in the older leaves. The results for Co or Mn in the ash parallel those for these elements in macerate extracts and "residues." The Ni content of ash and "residues" of the leaves have similar trends and reflect treatment, but the element was not detected in water extracts of the leaves, indicating that Ni is bound more closely in the tissues than the other metals examined.

The iron content of the ash of plants are given in Table VIII.

The leaves contain significantly higher amounts of iron than the stems. In the Pb or Mn treated plants the iron content of the older leaves is greater than the young leaves.

The total iron contents of the plants however, are not significantly changed by applying Co, Mn, Ni or Pb.

Chlorophyll Content.

The total chlorophyll contents of the young and old leaves respectively, as μg per gm fresh weight are presented in Table IX.

TABLE VIII.

THE IRON CONTENTS OF THE ASH OF TOMATO (STEM, YOUNG LEAF AND OLD LEAF)
IN RELATION TO THE APPLICATION OF CO, MN, NI AND PB RESPECTIVELY.

Metal	Plant Portion	Fe as $\log_{10}$ μg per gm dry matter	L.S.D.			Distribution of iron in plant portions
			0.001	0.01	0.05	
Pb	Stem	1.8602	0.2607	0.1897	0.1380	Old leaf > stem at 0.001 level
	Young leaf	2.0351				Old leaf > young leaf and stem at 0.01 level
	Old leaf	2.2823				Young leaf > stem at 0.05 level
Co	Stem	1.9326*	0.2825	0.2055	—	Old leaf > stem at 0.001 level
	Young leaf	2.2081*				Old leaf, young leaf > stem at 0.01 level
	Old leaf	2.3230*				
Ni	Stem	1.6971	0.2393	0.1740	0.1264	Old leaf, young leaf > stem at 0.001 level
	Young leaf	1.9764				Old leaf > young leaf at 0.05 level
	Old leaf	2.1457				
Mn	Stem	1.7472	0.0946	—	—	Old leaf > young leaf > stem at 0.001 level
	Young leaf	2.1485				
	Old leaf	2.2625				

*Values Fe, $\log_{10} \times 10$.

TABLE IX.

1st Sampling.

TOTAL CHLOROPHYLL CONTENT OF YOUNG AND OLD LEAVES OF TOMATO RESPECTIVELY, AS
 μG PER GM. FRESH WEIGHT.

Rate of metal applied m.eq. per pot	Leaf, Young (Y) Old (O)	Cu	Zn	Pb	Co	Ni	Mn	L.S.D. 0.05
0	Y	758.0	770.3	620.5	538.8	813.1	742.8	80.05
	O	577.7	644.6	498.2	519.3	681.6	553.2	N.S.
5	Y	739.7	663.3	712.2	568.6	602.2	654.1	80.05
	O	599.1	568.5	547.1	541.0	534.6	547.1	N.S.
10	Y	605.2	608.3	690.8	519.6	547.1	580.8	80.05
	O	550.2	626.6	504.4	489.1	519.6	571.6	N.S.

Old leaf, differences not significant.

Young leaf, rates of metal, significant at $P=0.05$.

Interaction between metals and rates was not significant.

EFFECTS OF RATES OF METAL (M.EQ. PER POT) ON CHLOROPHYLL CONTENT OF YOUNG LEAVES.

Rate of metal applied m.eq. per pot	0	5	10	L.S.D. P=0.05
Total chlorophyll as μg per gm. fresh weight	714.7	656.7	592.0	80.05
Order	1	2	3	

0 > 10 m.eq. metal P=0.05.

At the first sampling there appears to be more total chlorophyll in the young than in the old leaves. The chlorophyll content of the old leaves is not affected by metal application at this stage, but the young leaves show a significant decrease in the pigment at the 10 m.eq. of metal applied, coinciding with a significant reduction in water extractable iron. The interaction between metals and rates was not significant. At this stage of growth, iron chlorosis had just appeared in the young leaves of the Co and Ni treated plants, but became more marked later.

The chlorophylls A and B present in the control leaves were approximately 73.6% and 26.4% respectively, and in some of the iron induced chlorotic plants 62.0% and 38%.

Discussion.

The results of the factorial pot experiment are preliminary because the rates of metal applied to the soil, viz. 0, 5 and 10 m.eq. metal, were arbitrarily chosen, as previous information was not available on levels of metal required to induce toxicity effects in tomato in this soil. Moreover, the experiment was conducted out-of-doors so that there was little control of environmental factors. Nevertheless, certain results merit discussion. Co and Ni treatment induced marked iron deficiency symptoms, whereas those for Cu or Zn treated plants were slight. No visible effects were apparent in cultures receiving Pb or Mn. All metals other than Mn significantly reduced the fresh weight yields of plant "tops" at the second sampling.

A technique for the determination of metals in fresh plant tissues was developed as the ash analysis methods are time-consuming. Moreover, as there are usually no significant differences between the total iron contents of normal and iron deficient leaves (23, 25, 28), a fractionation of iron in the fresh plant tissue was attempted. The extraction method involves the maceration of small portions of leaves in a pyrex glass macerator with glass distilled water. The metals in the supernatant solution are passed through a column of glass wool and then exchanged on a specially prepared resin column e.g. Amberlite IR 100 or Zeocarb 215, so that the cations are retained and concentrated in the resin bed and freed from the anions. The metals were then discharged completely from the resin column with 2N.HCl and determined in the eluant. The metal content of the residues left after maceration was also determined. The ion exchange method is ideal for the concentration of metals, and removal of anions that may interfere with subsequent determination of the metals. Work is now in progress to separate the metals quantitatively on resin columns.

The most toxic metals, as far as plant growth is concerned, viz. Co and Ni, were present at lower levels in the leaf extracts than other metals which did not appear to affect the iron metabolism visibly. Thus Co and Ni exerted toxic effects at lower levels of metal, in the soil and plant, than other metals. Although Mn was present at very high levels in the plant extracts, it did not depress fresh weight yields or result in iron deficiency symptoms. These observations agree with previous work with Mn in sand culture (27) and soils (21).

The iron contents of the macerated extracts of young and old leaves respectively were significantly decreased as metal application was increased (young leaves $P=0.01$, old leaves $P=0.05$). The highly significant effect of metals on iron status of extracts of young leaves at the first sampling is of interest as chlorosis appears first in young foliage. The water soluble iron may be an indication of the so-called "active iron" present in plants, although Oserkowsky (23) used dilute HCl to extract "active iron" and showed that this was proportional to chlorophyll content. Lindner and Harley (16) and Hill and Lehmann (11) have used other methods for fractionating iron in plant tissues, so that further work is necessary to determine iron fractions which will reflect the severity of iron deficiency symptoms. In experiments described in this paper, the iron contents of the ash of tomato (stem, young and old leaves), were not significantly changed by the application of metals.

Total chlorophyll was greater in young than in old leaves. The young leaves at the first sampling, had significantly less total chlorophyll at the 10 m.eq. of metal applied, thus coinciding with their high metal content and low "extractable" iron and symptoms of iron induced deficiency in the Co and Ni cultures. The per cent. chlorophylls A and B was 73.6 and 26.4 respectively in the ether extract of chlorophyll. Although there is some evidence in this experiment of a correlation between water extractable iron and chlorophyll content, further work is necessary to determine whether this relationship is complicated by other factors. In particular, the relation of "active iron" to protein synthesis in the young leaves merits investigation.

Summary.

1. Tomato (var. Market King) was grown in a sandy loam soil derived from the Ross series (pH 5.5) in a factorial pot experiment. The effects of adding Cu, Zn, Pb, Co, Ni and Mn respectively at 0, 5 and 10 m.eq. rates to the soil were studied.

2. Induced iron deficiency symptoms appeared first in tomato given Co or Ni, then in the Cu or Zn treated cultures. The addition of Pb, although reducing yield of plant "tops" did not result in iron chlorosis. Mn did not visibly affect the iron status or yields of tops. Co and Ni in particular produced direct metal injury effects in addition to iron chlorosis.

3. A special technique was devised for the extraction of metals from leaf tissue with glass distilled water in pyrex glass macerators. The water extract after heat treatment and centrifuging was percolated through a clearing column of glass wool on to a cation exchange column (Zeocarb 215 or Amberlite I.R. 100). The metals were thus concentrated, freed from the anions and later eluted from the column with hydrochloric acid.

4. The metal content of the extracts and "residues" (left after maceration) of young and of old leaves was not related to the relative toxicity of the metals. Co and Ni which were very toxic to the plants, were present at significantly lower levels than Mn which was without effect on plant yields.

5. The iron level of the water extracts of young and of old leaves was significantly reduced by metal application. This iron fraction reflected iron chlorosis and may be a measure of "active iron" in the leaf tissues. The iron contents of the ash of leaves and stems were unaffected by metal application.

6. Total chlorophyll in the young leaves was significantly depressed at the highest level of metal application.

7. Further experiments are in progress to examine the biochemical effects of metal induced iron deficiency.

Acknowledgments.

I am grateful to Mr. H. M. Webber who assisted throughout with the experimental work, and to Miss Olive Woodhouse for help in recording the visual symptoms. Thanks are due to Mr. G. H. Jones who made the photographic records.

APPENDIX I.

Visual Symptoms in Tomato.

Excess Cobalt. Plants severely stunted, bright yellow chlorosis in young leaves appeared ten days after the first application of metal. Base of young leaflets first affected, spreading rapidly to leaf margins—iron deficiency type. Later brown necrotic areas develop intervenally, leaf margins fold up and become scorched, necrosis becomes severe, resulting in death of the leaflets. Simultaneously necrotic patches appear on petioles, stems and fruits, latter splitting longitudinally, flower trusses and calyces scorched, young fruitlets abscised. Growing points abort, and main stems die back. Symptoms spread rapidly to mid-stem leaves. Plate VII, Fig. 1.

Excess Nickel. Similar to excess cobalt effects, severe stunting, iron type chlorosis in young leaves rapidly followed by necrotic effects in leaf laminae, petioles and stems. Black necrotic areas start marginally as black spots and progress intervenally eventually covering the whole leaf. Necrosis more severe than in Co excess. Some yellow chlorosis later in old leaves, and finally death of the growing points. Symptoms spread quickly from young leaves and appeared in mid-stem leaves two weeks after first application of metal. Plate VII, Fig. 2.

Excess Copper. Growth stunted, slight yellow chlorosis at base of young leaflets, developed late, not as marked as for Co or Ni cultures. Slight necrosis usually marginal at first, later intervenal, purpling on underside of young leaflets and petioles. Older leaves upcurled margins, purpling intervenally.

Excess Zinc. Growth stunted, resembling Cu series in vigour, slight chlorosis and purpling in young leaves, appeared later than in Co or Ni cultures, older leaves upcurled margins, yellowing and purpling intervenally as for Cu cultures.

Excess Lead. Growth stunted, no chlorosis or necrosis of younger leaves; older leaves marked purpling intervenally, upcurled margins.

Excess Manganese. Growth not affected, plants vigorous; late in season older leaves, upcurled margins intervenal purpling and marked black venal necrosis starting marginally.

II. EFFECTS OF COPPER

AND ZINC ON CROP PLANTS GROWN IN VARIOUS SOILS.

Experimental.

Preliminary small scale pot experiments were made during 1949 with tomato, sugar beet and cauliflower in order to find the most suitable way of treating plants with heavy metals. The effects of both copper and zinc were studied in the 1949 experiments (A-C), whilst the effect of copper only was investigated in the 1950 experiment (D), using oat as the test crop.

In all experiments, except A, the clay pots used were painted with bitumen. The heavy metals were applied as solutions of the sulphates to the soil.

Experiment A.

Tomato (var. Stonor's M.P.) and sugar beet (var. Special Strain) were grown in a sterilised John Innes compost (pH 6.8) in ten and nine inch pots



Fig. 1. Cobalt toxicity in Tomato ; iron deficiency pattern in young leaves.



Fig. 2. Nickel toxicity in Tomato ; necrotic intervenal patches in young leaves, petiole and stem lesions, in addition to iron deficiency chlorosis.



Fig. 3. Tomato (Experiment C). Control (centre), and zinc-treated plants showing venel necrosis in the old leaves (left), and intervenal chlorosis in the young leaves (right).



Fig. 4. Sugar beet (Experiment B.2). Control (left), with copper-treated (centre) and zinc-treated (right) plants. Treated plants showing intervenal chlorosis.



Fig. 5. Cauliflower (Experiment C) grown in zinc-treated soil, showing intervenal chlorosis of one old leaf.

respectively, with one plant per pot. Six randomised blocks of 144 pots of tomato and 180 pots of sugar beet were arranged out of doors, and copper and zinc sulphate solutions applied at three rates, 0, 1 and 2. The tomato plants received in addition a supplement of ammonium, magnesium and potassium sulphates. Treatment of the plants with heavy metals began a month after potting out.

	<i>Tomato</i>		<i>Sugar Beet</i>	
	Cu	Zn	Cu	Zn
No. of applications	18	17	13	13
Total m.eq.* applied at rates				
1 and 2 respectively ..	563 1127	463 927	63 126	63 126

*m.eq.=milli-equivalents.

The amount of metal given increased as the experiment proceeded, the last four applications accounting for approximately 90% of the metal added.

Experiment B.

Tomato (var. Vetomold), sugar beet (var. Special Strain) and cauliflower (var. Majestic) were grown in a sterilised John Innes compost (pH 6.8), the tomato in six inch and the sugar beet and cauliflower in nine inch pots. The lay-out of the experiment was similar to that of experiment A, but treatment of the plants started a week after potting out.

	<i>Tomato</i>		<i>Sugar Beet</i>		<i>Cauliflower</i>	
	Cu	Zn	Cu	Zn	Cu	Zn
No. of applications	8	13	8	15	10	14
Total m.eq. applied						
at rates 1 and 2						
respectively ..	30 60 192 384	168 336 616 1232	204 408 492 984			

After harvesting the sugar beet plants, another batch of sugar beet seedlings were planted in the same pots in order to determine the effects of residual copper and zinc.

Experiment C.

Tomato (var. Vetomold), sugar beet (var. Special Strain) and cauliflower (var. Majestic) were grown in two composts (pH 5.3), derived from an acid soil of pH 6.4 and containing respectively 2/5 and 5/7 acid peat. The composts are designated D and E. For each crop there were 48 pots arranged in two randomised blocks in the glasshouse, with one plant per pot. The following rates 0, 1 and 2 of copper and zinc sulphates were given, with four pots per treatment, two of which contained compost D and the other two E.

	<i>Tomato</i>		<i>Sugar Beet</i>		<i>Cauliflower</i>	
	Cu	Zn	Cu	Zn	Cu	Zn
No. of applications	12	9 4	6 5	10 5	4	10 4
Total m.eq. applied						
at rates 1 and 2						
respectively* ..	31 47 75 95	72.5 120 84 120	47.5 95 87.5 115			

* Treatment in some cases decreased or discontinued, following wilting or death of the plants.

Experiment D.

Oat (var. S 84) was grown in a soil derived from the Ross series, pH approximately 5.0. Each pot contained about 20 oat seedlings; 192 pots were arranged in six randomised blocks in the glasshouse. Four rates of copper sulphate, 0, 1, 2 and 4 were given and the 14 applications made totalled 0, 17.5, 35 and 70 m.eq. Cu. Three weeks after starting treatment the plants

were sampled. Three samplings were made at five-day intervals. The plants were specially washed and divided into tops and roots and dried.

In this experiment the solution was applied to a small pot partly buried in the soil contained within the larger pot, in order to avoid the corrosive effect of copper sulphate solution observed in some of the earlier experiments.

Results.

Tables I and II show some of the effects obtained in Experiments A, B and C.

TABLE I.

THE EFFECTS OF COPPER ON TOMATO, SUGAR BEET AND CAULIFLOWER GROWN IN SOILS.

Crop Treatment	Total m.eq. applied per pot	Tomato	Total m.eq. applied per pot	Sugar Beet	Total m.eq. applied per pot	Cauliflower
A	563	100% dead ¹⁰⁺	126	No symptoms ⁹		
B	30	33% dead ⁶	168	* ⁹ A few plants chlorotic	204	17% dead ⁹
	60	67% dead	336	57% dead	408	33% dead
C Compost D	20	50% necrotic ⁶	60	⁶ 100% wilted, later chlorotic	47.5	25% dead ⁶
			120	100% dead	95	100% dead
C Compost E	31	No symptoms ⁶	60	⁶ Effects the same as in compost D	47.5	75% chlorotic ⁶
	47		120		95	100% dead

TABLE II.

THE EFFECTS OF ZINC ON TOMATO, SUGAR BEET AND CAULIFLOWER GROWN IN SOILS.

Crop Treatment	Total m.eq. applied per pot	Tomato	Total m.eq. applied per pot	Sugar Beet	Total m.eq. applied per pot	Cauliflower
A	163 463 927	¹⁰⁺ 25% chlorotic No symptoms 96% dead	126	⁹ No symptoms		
B	192	100% dead ⁶	616	* ⁹ A few plants chlorotic	492	17% showing purpling ⁹
			1232	60% dead	984	17% dead
C Compost D	47.5 95	75% necrotic ⁶ 75% dead	65	⁶ 50% chlorotic	100	No symptoms ⁶ 100% dead
			120	100% necrotic	115	
C Compost E	50	100% necrotic ⁶ 100% dead	65	⁶ Effects the same as in compost D	100	50% showing purpling ⁶ 50% dead
	95		120		115	

Notes.—

+ These figures refer to size of pot used in the experiment.

* Data for seedlings first transplanted into 3½" pots and later transferred to 9" pots.

Experiment A.

Statistical analysis of yield data of tomato fruit is given in Table III.

TABLE III.
STATISTICAL ANALYSIS OF YIELD DATA OF TOMATO FRUIT
IN RELATION TO COPPER OR ZINC TREATMENT.

Treatment. Total m.eq.—Cu	0	563	1127	L.S.D.
Mean of 6 blocks	17.782	6.271	5.156	1.87 at 0.001
Order	1	2	3	0.91 at 0.05

$P=0.001$. $0 > 563, 1127$ m.eq. Cu applied.

$P=0.05$. $563 > 1127$ m.eq. Cu applied.

Treatment. Total m.eq.—Zn	0	463	927	L.S.D.
Mean of 6 blocks	18.873	16.500	11.542	3.80 at 0.001
Order	1	2	3	1.84 at 0.05

$P=0.001$. $0, 463 > 927$ m.eq. Zn applied.

$P=0.05$. $0 > 463$ m.eq. Zn applied.

Application of 163 m.eq. zinc produced chlorosis in a number of tomato plants, as indicated in Table II, but the plants appeared to recover later.

Towards the end of the experiment, extracts were made of mid-stem leaves from treated and untreated tomato plants by means of the N.I.R.D. macerater (22). Nitrate was much higher in plants given copper and zinc than in control plants.

Experiment B.

(1) The sugar beet used in this experiment comprised two lots of seedlings. One batch was transplanted direct from the seed box into nine inch pots. The seedlings of the other batch were first transplanted into $3\frac{1}{2}$ " pots and later potted out in nine inch pots. The first group proved much more susceptible to heavy metal damage than the second.

(2) After the sugar beet in Experiment B.1 was harvested, two batches of plants similar to those used in Experiment B.1 were put in the same pots. In all cases damage was less severe than in Experiment B.1 indicating immobilisation of large proportions of the metals in the soil. A few of the zinc and copper treated plants developed a chlorosis of the iron type which was first observed on the older leaves. The application of a dilute ferrous sulphate solution to the affected leaves failed to correct the chlorosis. This may have been due to the application being made too late in the season. Root samples were analysed for K, Ca, Mg, P, nitrate N, Fe and Zn. There were no marked differences except for nitrate N which was higher in the zinc treated plants.

Experiment C.

Chemical tissue tests made at harvest time on tomato treated with copper and zinc respectively, showed that the treated plants had a lower K and a higher Mg content than controls. Iron occurred in all samples in traces only. Zinc was not detected in the foliage by chemical tissue tests.

Experiment D.

Statistical analysis of dry weights of roots and tops respectively showed no significant difference. Results of chemical tissue tests for K, Ca, Mg, P, nitrate N, Al, Mn and Fe were similar irrespective of treatment.

Statistical results obtained for "tops" first sampling, show that the copper content was significantly increased at the 0.001 level in the copper treated plants (Table IV).

TABLE IV.
STATISTICAL ANALYSIS OF CU CONTENT OF OAT "TOPS."

Treatment. Total m.eq. Cu applied per pot	0	17.5	35	70	L.S.D.
Mean of 6 blocks $\log_{10}$ (μg Cu/gm dry matter)	0.85	1.36	1.64	2.09	0.378 at 0.001
Order	1	2	3	4	0.257 at 0.01

P=0.001. $0 < 17.5, 35.0 < 70.0$ m.eq. Cu applied.

P=0.01. $0, 17.5 < 35.0$ m.eq. Cu applied.

The Ca, K, P, Mn and Fe contents were not significantly affected. The Mn contents varied from 310–563 $\mu\text{g./gm.}$ dry matter, so that the symptoms resembling "grey speck" were not due to Mn deficiency.

Discussion.

The experiments with tomato, sugar beet and cauliflower in John Innes' compost (pH 6.8) and with sugar beet in an acid compost (pH 5.3) indicate that copper is more toxic than zinc at similar milli-equivalent levels. The relative difference in toxicity of the two metals was not observed with tomato and sugar beet grown in the acid compost. Hewitt (10) also found copper to be more toxic than zinc, in sand culture experiments with tomato and sugar beet.

Much greater amounts of copper or zinc however, are required in soil than in sand culture to produce toxic effects in tomato and sugar beet. Thus only 1 m.eq. zinc per litre of basal nutrient solution induced toxicity effects in sugar beet grown in sand culture (10), whereas 1232 m.eq. zinc per pot John Innes' compost killed only 60% of the plants. Experiments B.1 and B.2 in the same compost, confirm that this difference may be due to metal fixation in the soil as the toxic effects produced in a second crop grown in the copper and zinc treated soils were much less. In agreement with these results, Lundblad *et al.* (17) and Gall and Barnette (8) have found that fixation of copper and zinc occurs readily in soils.

The effects observed in experiments A to C may be divided into two groups, namely: (1) wilting and necrosis produced by a lethal concentration

of available metal in the soil : (2) intervenal chlorosis of the iron or manganese deficiency type caused by either a very carefully regulated addition of the metal to the soil, or by a high concentration of metal in the soil, much of which has been "fixed."

The influence of experimental conditions on the effects produced is shown in the results of other workers. Thus Broyer and Furnstal (3) found that excess copper caused the old leaves of sugar beet growing in water culture to wither from the tips, whereas Hewitt (10) observed that excess copper in sand culture experiments with sugar beet resulted in intervenal chlorosis of the younger leaves as well as necrosis of the middle leaves.

As the chemical analysis of the oat samples is in progress, a discussion of the data will be deferred until a later Report.

Summary.

1. Tomato, sugar beet and cauliflower were grown in neutral and acid soils in randomised pot experiments. Large amounts of copper and of zinc sulphate solutions were applied to the soil at intervals during the season. Oat was grown in an acid soil and treated with excess copper only.
2. Copper was more toxic than zinc at similar milli-equivalent rates; tomato was more susceptible to injury by copper or zinc than sugar beet or cauliflower.
3. The amounts of copper and zinc respectively required to kill 50% of the plants ranged from 60 to 100, and 100 to 1200 m.eq. per pot. The corresponding figures for water or sand culture are much smaller owing to the metals being more readily available.
4. Toxicity symptoms usually appeared first in the older leaves and an iron type chlorosis was relatively infrequent. Treatment of a given crop with the same metal resulted in different symptoms depending on the soil, rates of application of metal, time of year and frequency of application.

Acknowledgments.

The author wishes to express his thanks to Dr. D. J. D. Nicholas for his continued advice and interest during the course of these experiments, and to Miss O. M. Woodhouse who recorded the results of the 1949 experiments and assisted in treating the plants.

APPENDIX II.

Visual Symptoms produced by excess Copper or Zinc.

Tomato.

Copper. A general yellow mottling developed in the older leaves and was followed by yellowing of the petioles. The leaf margins remained dark green. In experiment B the stems showed constrictions at soil level owing to prolonged contact with the applied copper solution. Two plants in experiment C, Compost D, developed grey necrotic areas near the margins and intervenally in the old leaves.

Zinc Symptoms developed in the older leaves which included a brown venal necrosis and uniform chlorosis. The younger leaves of some plants became chlorotic. (See Plate VIII, Fig. 3).

The veins near the margins of the older leaves in the copper and zinc treated plants frequently showed pink or purple colourations, especially on the dorsal surface.

Sugar Beet.

In the copper and zinc treatments in experiments B.1 and C, pinkish brown tinting developed intervenally in the older leaves and venal necrosis was frequent. Stunting of the roots also occurred. The symptoms produced by the two metals were similar.

A number of plants treated with copper and zinc in experiment B.2 showed a chlorosis of the iron type in the older leaves. Much necrosis was also present, and the treated plants were stunted. (See Plate VIII, Fig. 4).

Cauliflower.

Copper. Three kinds of symptoms were observed in the treated plants in experiment B, namely (1) grey interveinal areas on the older leaves; (2) interveinal chlorosis on the older leaves, and (3) constriction of the stem at soil level (cf. tomato). In experiment C, symptom (1) only was observed.

Zinc. A light green interveinal pattern, later followed by necrosis, was observed in the older leaves in experiment B. In experiment C the same effect was noted, but in addition several plants developed a yellowing in the older leaves. This did not resemble a typical iron deficiency chlorosis. (See Plate VIII, Fig. 5). Purpling of the stem and leaf axils occurred in a number of plants.

Oat. Two weeks after the first application of copper, both treated and untreated plants developed symptoms resembling "grey speck." Spare pots of oat plants were subjected to the following treatments respectively: (1) control; (2) 20 m.eq. Cu, applied to the soil; (3) 20 m.eq. Cu plus 100 p.p.m. Mo foliage spray; (4) 100 p.p.m. Mo foliage spray; (5) 0.2% MnSO_4 foliage spray. Five weeks later the plants in (1), (4) and (5) were healthy, those in (2) stunted, while most of the plants in (3) were dead.

Towards the end of experiment D, a few plants receiving the highest rate of copper developed bluish-green streaks in the old leaves.

REFERENCES.

- (1) AUDUS, L. J. (1946). *Nature*, **158**, 419.
- (2) BRENCHEY, W. E. (1938). *Ann. Appl. Biol.*, **25**, 671.
- (3) BROYER, T. C. and FURNSTAL, A. H. (1945). *Plant Physiol.*, **20**, 690.
- (4) CHAPMAN, H. D., LIEBIG, G. F. and VANSELOW, A. P. (1939). *Soil Sci. Soc. Amer. Proc.*, **4**, 196.
- (5) COMAR, C. L. (1942). *Ind. Eng. Chem. (Anal.)*, **14**, 877.
- (6) COTTON, M. (1930). *Bull. Torrey. Bot. Club*, **57**, 127.
- (7) ERKAMA, J. (1949). *Acta Chemica Scandinavica*, **3**, 850.
- (8) GALL, O. E. and BARNETTE, R. M. (1937). *Fla. Agr. Expt. Sta. Anni. Rept.*, 66.
- (9) HASELHOFF, E. (1893). *Landw. Jahrb.*, **22**, 862.
- (10) HEWITT, E. J. (1948). *Ann. Rep. Long Ashton Res. Sta.*, 66.
- (11) HILL, R. J. and LEHMANN, H. (1941). *Biochem. J.*, **35**, 1190.
- (12) HOLMES, R. S. (1945). *Soil Sci.*, **59**, 77.
- (13) HOPKINS, E. F., PAGAN, V. and RAMIREZ-SILVA, F. J. (1944). *J. Agric. Univ. Puerto Rico*, **28**, 143.
- (14) JONES, B. (1929). *Analyst*, **54**, 582.
- (15) LIEBIG, G. F., VANSELOW, A. P. and CHAPMAN, H. D. (1942). *Soil Sci.*, **53**, 341.
- (16) LINDNER, C. R. and HARLEY, C. P. (1944). *Plant Physiol.*, **19**, 420.
- (17) LUNDBLAD, K., SVANBERG, O. and EKMAN, P. (1949). *Plant and Soil*, **1**, 277.
- (18) MCGEORGE, W. T. (1923). *Soil Sci.*, **16**, 269.
- (19) MILLIKAN, C. R. (1947). *J. Austr. Inst. Agr. Sci.*, **13**, 4, 180.
- (20) MOSS, M. L. and MELLON, M. G. (1942). *Ind. and Eng. Chem. (Anal.)*, **14**, 862.
- (21) NICHOLAS, D. J. D. (1949). *J. Hort. Sci.*, **25**, 1, 60.
- (22) ——— (1951). *Mimeo publication, Long Ashton Res. Sta.*
- (23) OSERKOWSKY, J. (1933). *Plant Physiol.*, **8**, 449.
- (24) SANDELL, E. B. (1944). "Colorimetric Determination of Traces of Metals." *New York Interscience Publishers, Inc.*
- (25) SOMERS, I. I. and SHIVE, T. W. (1942). *Plant Physiol.*, **17**, 582.
- (26) TWYMAN, E. S. (1946). *New Phytol.*, **45**, 18.
- (27) WALLACE, T., HEWITT, E. J. and NICHOLAS, D. J. D. (1945). *Nature*, **156**, 778.
- (28) WALLACE, T. and HEWITT, E. J. (1946). *J. Pomol. and Hort. Sci.*, **22**, 153.
- (29) WILLIS, L. G. and PILAND, J. R. (1936). *J. Agric. Res.*, **52**, 467.

A NOTE ON THE USE OF TETRAMETHYLDIAMINODIPHENYLMETHANE FOR DETERMINING SMALL AMOUNTS OF MANGANESE IN PLANTS.

By D. J. D. NICHOLAS and D. J. FISHER.

The determination of manganese in the ash of plants is usually done by oxidising the element to potassium permanganate with bismuthate (1, 11) or periodate (5, 6, 8). Formaldoxime has also been used to detect Mn in plants (5, 6, 7, 9). These methods however, are not sufficiently sensitive to determine small amounts accurately, viz., less than 10 μg Mn per gm. dry matter. 4:4'-tetramethyldiaminodiphenylmethane or "tetrabase" is a sensitive and specific reagent for Mn (7, 10). It has been used to determine low levels of the element in extracts of plant tissues (4, 6) and in sea water (3) which were not detected by other chemical methods. Cornfield and Pollard (2) have recently used "tetrabase" to determine Mn in plant material and soil extracts. Their method is based on comparisons of the green colour produced after 10 min. of adding the test reagents.

In this paper a method is described for determining 0.05 to 0.4 μg Mn in acid digests of plant material. An aliquot of the plant digest is buffered with acetate solution, cooled in ice and the blue colour formed exactly 4 min. after adding the reagents, is determined with a Unicam spectrophotometer, Biochem or Eel absorptiometers. It is shown that under condition of test the method is specific for Mn. The duplication of the results is close and the agreement with the periodate method is good. Lower levels of the element are more readily determined by "tetrabase," which is more sensitive than the periodate method. Moreover, the "tetrabase" test does not require heat treatment and is quicker in operation.

Method.

Reagents.

Tetrabase : 1 gm., 4:4' tetramethyldiaminodiphenylmethane dissolved in 100 ml. of redistilled acetone. A fresh solution is made up daily and is stored in a refrigerator when not in use.

Acetic Acid : redistilled glacial acetic acid.

Potassium periodate : recrystallised; saturated solution in glass distilled water and stored in a refrigerator.

Acetate buffer : 100 gm. sodium acetate (anhydrous) or 166 gm. sodium acetate (trihydrate) are dissolved in about 500 ml. of distilled water. The solution is freed from Mn as follows : add 0.2 gm. Na_2HPO_4 , 2 gm. $\text{Ca}(\text{NO}_3)_2$ and 10 gm. CaCO_3 , mix well and autoclave for $\frac{3}{4}$ hr. at approximately 120° C. After cooling the solution is filtered under pressure through a Buchner funnel into a pyrex conical flask, using a Whatman 42 filter paper. 5 ml. of this solution is tested with "tetrabase" to confirm the complete removal of manganese. If Mn is present, the purification procedure is repeated. 30 ml. of redistilled glacial acetic acid are added and the solution made up to 1 litre with glass distilled water.

Standard Manganese Solutions.

A standard solution containing 20 μg Mn per ml. is made up as follows : 1.4385 gm. potassium permanganate is dissolved in 200 ml. of distilled water and made up to 1 litre. To 20 ml. of this solution is added 5 ml. of $2\text{N.H}_2\text{SO}_4$ and a few crystals of sodium sulphite and it is boiled for a few minutes to remove excess SO_2 . After cooling, the solution is made up to 500 ml. with glass distilled water.

A further dilution of 1 in 20 ml. is made with distilled water (1 μg Mn per ml). A final dilution of 1 in 10 is made with the acetate buffer so that the pH of the final solution approximates to 4.8 and contains 0.1 μg Mn per ml.

Preparation of Calibration Diagrams.

In matched tubes, 6×1.5 cm., which have been thoroughly cleaned in a chromic sulphuric acid bath, rinsed with glass distilled water and redistilled acetone and dried, are put 5 ml. of standard solutions containing 0.05, 0.1, 0.15, 0.2, . . . 0.4 μg Mn respectively. The tubes are put in crushed ice for 2 min. and the following reagents are dispensed from burettes in the following order : 0.5 ml. acetic acid, 0.5 ml. potassium periodate and 0.1 ml. of "tetrabase."

The solution is well shaken after addition of each reagent. The test tube is kept in ice for *exactly* 4 min. after adding the reagents, and the blue colour which develops is measured with a Unicam D.G. spectrophotometer, Biochem or Eel absorptimeters. It is important to develop each test solution singly.

Acid Digests of Plants.

Between 2 and 5 gm. of oven dry plant material is weighed in a 400 ml. pyrex beaker. 15 ml. of nitric acid A.R. (S.G. 1.42) per gm. of dry matter is used and the material digested on a hot plate for an hour until the volume is approximately 10 ml. After cooling, 2 ml. of perchloric acid (70% W/V) per gm. of dry matter are put in the beaker and digestion continued to the "incipient" dryness stage when all the organic matter and excess acid have been removed (5, 6). The digest is cooled and 100 ml. of glass distilled water added and the solution boiled for 30 min. to dissolve and hydrolise the mineral elements. After cooling, the digest is filtered through a No. 42 Whatman paper into a 250 ml. graduated flask and made to volume with distilled water. Appropriate aliquots of this solution are taken for test, viz., 1 or 2 ml. of digest solution are pipetted into a matched tube and the volume made up to exactly 5 ml. with the acetate buffer solution. The test is made as for the standard Mn solutions.

Results.

Calibration of Manganese Standard Solutions.

The percentage transmission of the blue colour was determined for 0.3 μg Mn, exactly 4 min. after addition of reagents, by means of the Unicam D.G. spectrophotometer. The results are shown in Fig. 1. The maximum transmission occurred between the following wavelengths $m\mu$ 580 and 600. The Mn standards were initially determined at a wavelength of $m\mu$ 590, but the calibration diagram covered a very narrow range, from 0.01 to 0.1 μg Mn. As this range was too small for Mn in plant digests, a calibration graph was prepared at a wavelength of 520 $m\mu$ from 0.05 to 0.4 μg Mn as shown in Fig. 2. A graph prepared with a Biochem absorptimeter for a similar range of Mn using red filters is also shown in Fig. 2.

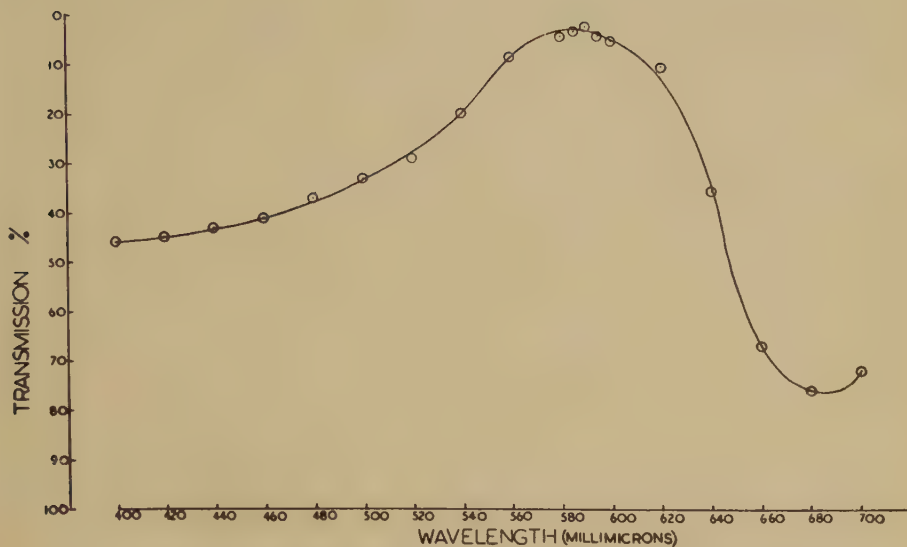


Fig. 1. Transmission diagram for $0.3 \mu\text{g}$ Mn at various wavelengths using the "tetrabase" method. Blue colour determined, exactly 4 min. after addition of reagents, with the Unicam D.G. spectrophotometer.

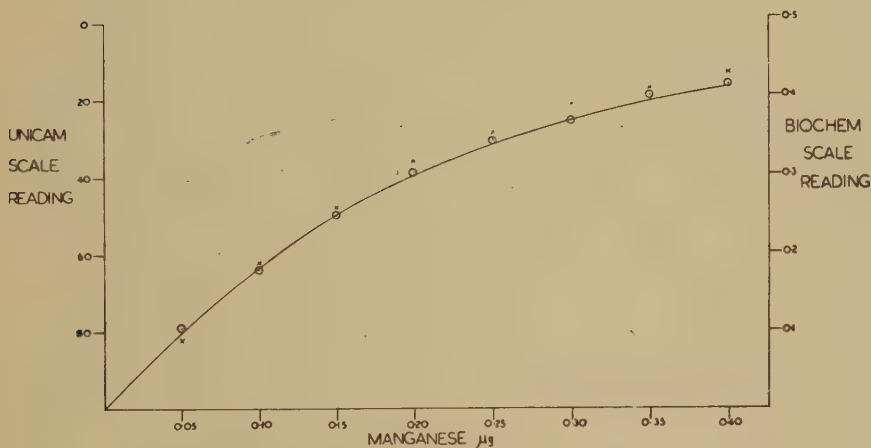


Fig. 2. Calibration diagrams for $0.05 \mu\text{g}$ to $0.4 \mu\text{g}$ Mn using the "tetrabase" method prepared by means of the Biochem absorptiometer and Unicam D.G. spectrophotometer respectively.

0—0; Spectrophotometer readings at $m\mu$ 520.

X—X; Biochem absorptiometer readings.

Manganese Status of Crops.

The Mn status of the mid-stem leaves of oat was determined in duplicate with "tetrabase" using the D.G. spectrophotometer. The results are given in Table I.

TABLE I.

DUPLICATE ANALYSES FOR MN IN ACID DIGESTS OF OAT (MID-STEM LEAVES) USING THE "TETRABASE" METHOD. (UNICAM D.G. SPECTROPHOTOMETER).

Dry Weight in gm.		5-19	5-19	4-97	5-10	5-17	5-18	5-19	5-12	5-16	5-11	5-07	5-18
Mn as μ g. per gm. dry matter	"Tetrabase" method	11	13	13	10	11	10	9	9	10	10	15	13
	"Tetrabase" method	11	13	14	11	11	10	9	9	9	10	15	11

Mean of 12 values.

"Tetrabase"
11-16

"Tetrabase"
11-08

Significant Differences.

P = 0-05
0-084

P = 0-01
0-118

$$0.8 < P < 0.7.$$

Differences between duplicate results are not significant.

There are no significant differences ($n = 12$, $t = 0.38$) between the Mn results for the same digests, so that the agreement of duplicate analyses is close. Moreover, the recovery from plant digests of added Mn was within the limits of experimental error.

In Table II a comparison is made between the results of the periodate and the "tetrabase" methods for Mn in the same acid digests of oat leaves.

TABLE II.

A COMPARISON BETWEEN THE PERIODATE AND "TETRABASE" METHODS FOR DETERMINING THE MN STATUS OF OAT (MID-STEM LEAVES) (BIOCHEM ABSORPTIOMETER).

Dry Weight in gm.		5-48	4-43	4-79	4-40	4-86	5-36	4-86	4-86	5-26	5-14	5-48	5-33
Mn as μ g. per gm. dry matter	Periodate method	3	7	3	3	3	2	13	10	2	5	5	1
	"Tetrabase" method	8	5	4	7	7	3	8	19	4	7	9	6

Dry Weight in gm.		5-35	5-31	5-35	5-41	5-36	5-39	5-37	5-40	5-39	5-52	5-50	5-33	5-37
Mn as μ g. per gm. dry matter	Periodate method	2	2	4	2	3	9	7	3	2	4	5	4	3
	"Tetrabase" method	6	11	8	7	6	7	10	8	6	4	7	7	7

Mean of 25 values.

"Tetrabase"
5-54

Periodate
4-28

Significant Differences.

P = 0-05
3-508

P = 0-01
4-754

$$0.2 < P < 0.1.$$

Differences between the results of the two methods are not significant.

The statistical results show that there are no significant differences between the results of the two methods ($n = 25$, $t = 1.70$) so that either may be used to determine the Mn status of plant digests.

In Table III a further comparison is made between the two methods for determining the Mn status of oat using the Unicam D.G. spectrophotometer ($n = 65$, $t = 1.52$). There are no significant differences between the results of the two methods.

TABLE III.

A COMPARISON BETWEEN THE MN STATUS OF OAT (MID-STEM LEAVES) DETERMINED BY PERIODATE AND "TETRABASE" METHODS (UNICAM D.G. SPECTROPHOTOMETER).

Dry Weight in gm.		5.05	5.73	5.54	5.55	5.66	4.23	5.63	5.53	5.15	5.07	5.15	5.16	5.13	5.12	5.19	5.10
Mn as μg per gm. dry matter	Periodate method	8	7	7	7	6	10	7	3	8	7	10	6	12	6	12	11
	"Tetrabase" method	10	12	10	10	8	15	11	12	13	14	19	14	17	12	15	15

Dry Weight in gm.		4.35	4.65	5.10	5.14	5.14	4.84	5.04	5.13	5.11	5.11	5.06	5.09	5.09	5.05	5.11	5.09
Mn as μg per gm. dry matter	Periodate method	9	4	7	7	7	15	5	7	5	3	10	2	10	2	2	3
	"Tetrabase" method	17	13	11	9	12	20	9	11	8	7	6	7	10	6	7	11

Dry Weight in gm.		5.17	5.22	5.14	5.13	5.05	5.08	5.08	5.19	4.82	5.14	5.19	5.10	5.22	4.50	4.43	5.26
Mn as μg per gm. dry matter	Periodate method	10	10	7	13	14	2	10	9	17	13	9	15	7	8	8	7
	"Tetrabase" method	11	13	16	13	19	8	11	13	21	11	10	20	14	10	11	11

Dry Weight in gm.		5.21	5.24	5.14	5.18	5.04	5.19	5.19	4.97	5.10	5.17	5.18	5.19	5.12	5.16	5.11	5.07	5.18
Mn as μg per gm. dry matter	Periodate method	11	8	3	2	2	12	13	15	10	10	10	8	8	10	8	17	14
	"Tetrabase" method	13	12	11	15	15	11	13	13	11	11	10	10	9	10	10	15	12

Mean of 65 values.

"Tetrabase"
12.05

Periodate
8.35

Significant Differences.

P = 0.05
3.04

P = 0.01
4.04

$0.2 < P < 0.1$.

Differences between the results of the two methods are not significant.

TABLE IV.

A COMPARISON BETWEEN THE PERIODATE AND "TETRABASE" METHODS FOR DETERMINING THE MN STATUS OF OAT (MID-STEM LEAVES) (BIOCHEM ABSORPTIOMETER).

Dry Weight in gm.		6.00	4.84	4.59	4.92	4.54	5.43	5.17	4.83	4.99	4.70	5.52	6.21
Mn as μg per gm. dry matter	Periodate method	230	117	127	320	376	296	481	573	628	32	31	941
	"Tetrabase" method	254	116	111	317	387	267	508	509	811	43	24	896

Mean of 12 values.

"Tetrabase"
354.0

Periodate
346.0

Significant Differences.

P = 0.05
2.97

P = 0.01
4.19

$0.3 < P < 0.2$.

Differences between the results of the two methods are not significant.

In Table IV a wide range of Mn values have been determined by the two methods in plant digests. The correlation is again close ($n = 12$, $t = 1.35$). There are no significant differences between the Mn status as determined by the two methods.

The Mn status of other crops including potato was determined by the two methods and the agreement is within experimental error.

Discussion.

During the course of an investigation on the relation between the Mn status of oat and final yields, it became necessary to determine small amounts of Mn in plant digests. The periodate (5, 6, 8), and formaldoxime (5, 6, 7, 9) methods are not sufficiently sensitive to detect deficiency levels of Mn in plants. A "tetrabase" test was developed to determine the element in aliquots of nitric-perchloric acid digests of plant material from 0.1 to 0.4 μg Mn. Tetramethyldiaminodiphenylmethane which has been used to determine deficiency levels of Mn in tissue extracts of plants (4, 6) is specific for Mn under condition of test. Moreover, statistical results show that recovery of Mn added to plant digests was within the limits of experimental error. The "t" test made on duplicate "tetrabase" analyses and on the results of the "tetrabase" and periodate methods indicate close agreement. The blue colour developed exactly 4 min. after adding the reagents was satisfactory for comparison and was superior to the fluorescent green and red colours which are formed later. It is important to make the comparison in the absorptiometer after exactly the specified time as the blue colour is unstable.

Summary.

1. A sensitive method is described for determining small amounts of Mn in acid digests of plants with tetramethyldiaminodiphenylmethane or "tetrabase."

2. The blue colour of the test solution which was developed in an icebath was determined by means of either the D.G. spectrophotometer (wavelength $m\mu$ 520) or absorptiometers (red filters) exactly 4 min. after adding the reagents. The effective range is from 0.1 to 0.4 μg Mn.

3. Under condition of test the "tetrabase" is specific for Mn. Duplicate analyses and recovery of added Mn were within the limits of experimental error.

4. A comparison between the results of the "tetrabase" and periodate methods for a range of plant digests showed close agreement. The "t" test showed no significant differences between the results of the two methods whether determined by absorptiometers or the D.G. spectrophotometer.

5. The specificity, sensitivity and ease of determining low levels of Mn by "tetrabase" makes it preferable to the periodate or formaldoxime methods for routine use.

REFERENCES.

- (1) COLLINS, W. D. and FOSTER, M. D. (1924). *Ind. Eng. Chem.*, **16**, 586; *Analyst*, 1924, 395.
- (2) CORNFIELD, A. H. and POLLARD, A. G. (1950). *J. Sci. of Food and Agr.* **1**, No. 4, 107.
- (3) HARVEY, H. W. (1949). *J. Mar. Biol. Assoc.*, **28**, 155.
- (4) NICHOLAS, D. J. D. (1946). *Nature*, **157**, 695.
- (5) NICHOLAS, D. J. D. (1948). *J. hort. Sci.*, **24**, 3, 72.
- (6) NICHOLAS, D. J. D. (1949). *J. hort. Sci.*, **25**, 1, 60.
- (7) NICHOLAS, D. J. D. (1951). *Mimeo publication of Long Ashton Research Station*.
- (8) RICHARDS, M. B. (1930). *Analyst*, **55**, 554.
- (9) SIDERIS, C. P. (1937). *Ind. Eng. Chem. (Anal.)*, **9**, 445 and *Analyst*, 1940, 476.
- (10) VOGEL, A. I. (1939). 'A textbook of Qualitative Chemical Analysis'. Longmans.
- (11) WILLIAMS, G. W. M. (1949). 'Trace Elements in Food'. Chapman and Hall, Ltd.

THE INSECTICIDAL ACTION OF GAMMA-HEXACHLOROCYCLOHEXANE.

By D. WOODCOCK.

The insecticidal action of the mixture of hexachlorocyclohexanes ("benzene hexachloride") obtained by the nuclear chlorination of benzene in the presence of ultra-violet light, was reported by Slade (1) to be due almost exclusively to the γ -isomer.

Mention of the isosteric resemblance between the hexachloro- and hexahydrocyclohexanes (the inositols) had been made earlier by Dr. Olive Mooney and the structure of meso-inositol was investigated by Posternak (2) and by Dangschat and Fischer (3) who agreed that the molecular configuration was very similar to that of γ -hexachlorocyclohexane, though all attempts by Dacey, Colucci and Kirkwood (4) to convert the isomers to inositols were unsuccessful.

Slade (1945) suggested that the insecticidal superiority of the γ -isomer was due to competition with the essential metabolite meso-inositol. This hypothesis received some support from the work of Kirkwood and Phillips (5) who studied the effect of the addition of the various isomers to media used for growing the Gebrüder-Meyer strain of *Saccharomyces cerevisiae*—an organism which must have meso-inositol for normal growth. The inhibition of growth was slight for the α and β isomers and more marked for the δ , but in no case was the inhibition reversed by the addition of inositol. With the γ -isomer, however, growth was inhibited by a concentration of 50 $\mu\text{g./ml.}$ and progressively reversed by the addition of inositol, though reversal was not complete. Further support was given by the work of Buston et al. (6) with *Nematospora Gossypii* in which the γ -isomer alone curtailed growth. On the other hand, attempts to show antidotal effects from the administration of inositol to insects poisoned by γ -hexachlorocyclohexane were unsuccessful. Thus Dresden and Krijgsman (7) found that the L.D. 50 of injected water-oil emulsions of the γ -isomer to *Periplaneta americana* was not altered by the simultaneous administration of the same or even twice the amount of meso-inositol.

If the essential metabolite concept is valid then compounds isosteric with the γ -isomer should also be toxic. Synthesis of hexamethylcyclohexane was undertaken in order to investigate this possibility.

Following preliminary experiments with hexaethylcyclohexane by Stringer and Woodcock (8) the hexamethyl compound was obtained similarly by reduction of the corresponding hexasubstituted benzene in the presence of Raney nickel (cf. Djakowa et al. (9)). The stereoisomeric mixture obtained from this reduction which probably contained some of the methyl isostere of γ -hexachlorocyclohexane, was completely non-toxic when tested as a contact insecticide against *Calandra granaria* (15). In view of the results of Metcalf (10) who was unable to show that the presence of any of the other isomers alone or in combination had any effect on the toxicity of the γ -isomer to *Heliothrips haemorrhoidalis*, it is unlikely that γ -hexachlorocyclohexane acts as a competitive metabolite to inositol.

Final downfall of the "inositol theory" came after the completion of this work. Dutch workers at Utrecht (11) announced the elucidation of the structure of the γ -isomer of hexachlorocyclohexane. X-ray analysis showed

the cyclohexane ring to be of the "chair" form and not planar as had often been assumed, and the configuration of the chlorine atoms to be of the $\epsilon\epsilon\epsilon\epsilon\epsilon\epsilon$ type in the nomenclature of Hassel (12). The γ -isomer is thus not isosteric with inositol, the -OH groups of which are known to be oriented $\epsilon\epsilon\epsilon\epsilon\epsilon\epsilon$. This important result was verified by Hassel and co-workers (13) who also established the configurations of the remaining isomers using a vapour-phase diffraction method and additional confirmation was furnished by the dipole moment data given by Hetland (14) for the five known isomers.

Whether the high insecticidal value of the γ -isomer is due to a stereo-configuration which allows a specific attack on some other physiological process has never been suggested and must not be considered impossible, though the work of Dresden and Krijnsman (loc. cit.) has made it clear that the difference in activity of the isomers is not due to different rates of cuticle penetration.

Experimental.

Hexaethylcyclohexane.—Hexaethylbenzene (B.D.H. 10g.) was dissolved in cyclohexane (80 ml.) and hydrogenated in the presence of Raney nickel at 200–250° and 120–130 atmos. After 20 hours, the reaction mixture was allowed to cool and the filtered solution evaporated. Distillation of the colourless residue gave two fractions :

2.7 g. b.p.	133°/15 mm.
6.6 g. b.p.	138–145°/15 mm.

Redistillation of the second gave a mid-fraction, b.p. 139–141°/14.5 mm., $n_D^{15}=1.4741$ (Found : C, 85.9 ; H, 13.9. Calc. for $C_{18}H_{36}$; C, 85.7 ; H, 14.3%). Koch and Steinbrink (Brennstoff Chemie, 1938, 19, 277, ibid. 407) give b.p. 153.3–155°/16 mm.

Hexamethylcyclohexane.—Hexamethylbenzene (5 g.) dissolved in cyclohexane (50 ml.) was hydrogenated at 270–280° under 80 atmos. pressure for 18 hours in the presence of Raney nickel. The product, isolated as described above, was a colourless liquid (3.7 g.) b.p. 87–88°/12 mm. Redistillation for analysis gave a fraction b.p. 103–4°/24 mm. $n_D^{15}=1.4654$ (Found : C, 85.5 ; H, 14.5. Calc. for $C_{12}H_{24}$; C, 85.7 ; H, 14.3%). Djakowa et al. give b.p. 214–216°, $n_D^{20}=1.4638$.

Summary.

Hexamethyl- and hexaethylcyclohexanes have been synthesised but have proved inactive as contact insecticides against *Calandra granaria*.L. The inadequacy of the Inositol Theory as an explanation of the insecticidal activity of γ -hexachlorocyclohexane, is discussed.

Acknowledgments.

My grateful thanks are due to Professor W. Baker, F.R.S., of the Chemistry Department for his generous provision of laboratory facilities, and to the Director, C. R. L. Teddington, for a gift of hexamethylbenzene.

REFERENCES.

- (1) SLADE, R. E., Chem. and Ind. 1945, **64**, 314.
- (2) POSTERNAK, T., Compt. rend. Soc. Phys. hist. nat. Geneve (1941), **58**, 44.
- (3) DANGSCHAT, G. and FISCHER, H. O. Naturwissenschaften (1942), **30**, 146.
- (4) DACEY, J. R., COLUCCI, J., and KIRKWOOD, S., unpub. data. Canadian National Research Council, 1944.

- (5) KIRKWOOD, S., and PHILLIPS, P. H., J. Pharmacol. & Expt. Therap. (1946), **87**, 375.
- (6) BUSTON, H., JACOBS, S. and GOLDSTEIN, A., Nature (1946), **158**, 22.
- (7) DRESDEN, D. and KRIJSMAN, B., Bull. Entomol. Res. (1948), **38**, 575.
- (8) STRINGER, A. and WOODCOCK, D., Chem. & Ind. (1948), 110.
- (9) DJAKOWA, LOSOWOI and STEPANOWA, Chem. J. Ser. A.J. allg. Chem. (1937), **7** (69), 722.
- (10) METCALF, R. L., Journ. Econ. Entomol. (1947), **40**, 522.
- (11) VAN VLOPEN, KRUSSINK, STRIJK and BIJVOET, Nature (1948), **162**, 771.
- (12) HASSEL, O., Tids. Kjemie Bergvesen. Met. (1943), **3**, 32.
- (13) HASSEL, O. et al., Research (1949), **2**, 248.
- (14) HETLAND, ACTA. Chem. Scand. (1948), **2**, 678.
- (15) STRINGER, A., Ann. Appl. Biol. (1949), **36**, 213.

A NOTE ON THE CONTROL OF LEAF CURLING PLUM APHID BY A D.D.T. EMULSION.

By H. G. H. KEARNS and N. G. MORGAN.

Leaf Curling Plum Aphid (*Brachycaudus helichrysi* Kltb.) is annually controlled by the application of tar oil or D.N.C. petroleum emulsions. Generally it is recommended that these winter washes should be applied not later than mid-January to reduce the risk of bud damage, especially on the loose budded varieties such as Victoria. The D.N.C. petroleum washes (3 per cent oil) also provide some control of red spider eggs, but the best results on apples are only obtained if the application is made in March which is too late for plums. In any event it is doubtful if the control obtained by the petroleum oil warrants the expense, and much better results are obtained by spraying in spring with contact washes.

Observations in the West Country over a number of years have shown that a very few aphid eggs on an unsprayed tree is sufficient to result in a devastating infestation. This is explained by the prolific breeding of the aphides and the extensive leaf curling which can result from the punctures of a few aphides. The date of egg hatch, in contrast to that of the apple species, is well before bud burst and it may be from the third week in January until mid February. The stem mother aphid, which is brown in colour and not unlike a spider, feeds on the bud scales and the first batch of viviparous nymphs is produced in about three weeks. These nymphs generally wander along the shoots and one or more are found on the bud scales and at the base of the flower buds awaiting their opening. From the stage when the buds show a white tip the young aphides feed and become a yellowish green colour. From this time the population increases rapidly and a second brood of viviparous nymphs can be produced in seven days. As soon as the flowers open and the leaves begin to develop the aphides commence their feeding and the leaves develop a curl. Inside the curling leaves rapid reproduction occurs and the aphides spread throughout the tree and in many cases practically every leaf on the tree is curled and stunted in growth. Winged aphides may leave the tree as early as the third week in March but most leave in late April and May and then the tree slowly recovers from the attack. If red spider is present in numbers and the season favourable to their development then the foliage suffers a second devastating attack. These attacks reduce the fruiting capacity of the tree for one or two seasons.

Early flowering varieties such as Early Laxton are particularly liable to severe damage as their early development allows the population of aphides to build up rapidly. Late flowering varieties, such as Belle de Louvain are less likely to suffer.

Control by D.D.T.

In 1948-1950 a number of small field trials were carried out to determine the value of D.D.T. for the control of leaf curling plum aphid.

The observations on the movements of the aphides suggested that they were most vulnerable to contact sprays up to bud burst and laboratory trials showed that the aphides were readily killed by D.D.T. benzol emulsion. Unfortunately, application at this stage on most varieties is too early for the control of winter moth larvae and Plum Sawfly. Small field trials were carried out in 1948 and 1949 to determine if the aphides could be controlled by D.D.T. at the split "cot" stage and when a number of the leaves were curling.

The D.D.T. was prepared as a benzol-sulphite lye emulsion and applied at a concentration of 0.05 per cent D.D.T. and 0.2 per cent benzol. The emulsion alone had insufficient wetting properties so "Shellestol" was added to the diluted wash, two concentrations of wetter, namely 0.25 and 0.5 per cent being tested. The washes were applied to the trees until prolonged run off had occurred. Despite this careful spraying insufficient numbers of the aphides within the curled leaves were killed and a devastating infestation resulted. An examination of the distribution of the spray fluid showed that the mechanical obstructions caused by the partially curled leaves made it impossible to wet the complete surface of the leaves. Similar experiments substituting the D.D.T. by nicotine were equally unsatisfactory in controlling the aphides. Spraying an infested tree continuously from all angles failed to secure sufficient entry of the wash into the curled leaves.

In view of this failure to control the aphides after the blossoming stage due to the curling of the leaves, D.D.T. was applied in the following season just before bud burst.

In 1950 a row of 80 trees approximately 12 ft. high, including the varieties Early Laxton, Victoria, Pershore Egg, Burbanks, Laxton Bountiful and Belle de Louvain, were divided into blocks with controls to each variety. The trees were sprayed on the 6th March with the D.D.T. emulsion described above and one quart of "Shellestol" per 100 gallons of wash.

The control of aphides was so complete that not a single leaf was curled whereas on the unsprayed control trees, excluding Belle de Louvain, it was difficult to find an uncurled leaf. At the bud burst stage there was no difficulty in obtaining a complete wetting of the bark: large droplets of the emulsion collected around and in the axils of the buds and the aphides were completely immersed so the only chance of survival would be by "misses" due to bad spraying.

Summary.

- (1) A D.D.T. benzol emulsion, 0.05 per cent D.D.T., 0.2 per cent benzol and 0.25 per cent "Shellestol" wetter applied after the flowering stage failed to control Leaf-curling Plum Aphid. An increase in the concentration of the wetter provided no obvious increase in control.
- (2) The same D.D.T. emulsion applied at bud burst provided a complete control of the aphid.

Acknowledgment.

Thanks are due to Mr. A. G. Wilkins for his careful observations on the aphides.

APPLE AND PEAR SCAB SPRAYING EXPERIMENTS AT LONG ASHTON, 1949 AND 1950.

By R. J. W. BYRDE and R. W. MARSH.

Introductory.

In the search for fungicides capable of controlling apple scab with less risk of spray injury than is entailed in using the standard lime sulphur programme, promising results were recorded in 1947 (3) for preparations of glyoxalidines. However, in trials made in 1948 (4) there were indications that these materials might cause slight injury to foliage of Cox's Orange Pippin, and there have also been recent records of phytotoxic effects of glyoxalidines in U.S.A. (5). Further trials were therefore planned, at Long Ashton and elsewhere, to investigate the effects on apples of varying the strengths and formulations of glyoxalidine sprays. A preliminary account of the results obtained at East Malling in 1949 has been published by Kirby (2). At Long Ashton, the trials were extended in 1949 to include both apple and pear varieties. Also, in 1950, preliminary trials were made on apples with three other newly-introduced fungicides, which had shown promise in laboratory bioassays (1) and other tests (5).

The results of two years' experiments are presented in the same paper to facilitate a comparison between the effects of the warm, dry summer of 1949 and those of the cool, wet summer of 1950.

Plots.

The apple trials were carried out on plot 23 in the Long Ashton plantations. The trees used were planted in 1935 and included two main blocks (2 acres each) of Cox's Orange Pippin and Worcester Pearmain, two 30-tree blocks of the same varieties, and 10 trees each of Lane's Prince Albert and Stirling Castle. The whole plot was originally set out at 15 ft. on the square, but, except in the main Worcester block, alternate trees were subsequently removed leaving a 21 ft. diagonal plant.

The pears used comprised single 25-tree rows of William's Bon Chrétien and Doyenné du Comice planted at 15 ft. intervals. The original trees were 15 years old but there were many younger replacements.

Materials.

The fungicides tested were compounded as follows :—

Lime-sulphur, as a dilute solution of the commercial concentrate.

Experimental Fungicide 341-C, as a water-miscible liquid stated to contain :—

2-heptadecyl glyoxalidine acetate	32% (by wt.)
2-pentadecyl glyoxalidine acetate	16% (by wt.)
2-heptadecenyl glyoxalidine acetate	2% (by wt.)
Isopropanol	50% (by wt.)

It was used at the rate of 1 quart or 1 pint per 100 gallons; 8 ozs. hydrated lime were added to each 100 gallons of diluted spray, and the mixture well agitated.

Experimental Fungicide 341-SC. This is a modification of the 341-C formulation stated to consist essentially of 34% 2-heptadecyl glyoxalidine

acetate and 66% isopropanol, the heptadecenyl derivative being almost entirely absent.

Phygon "XL," as an aqueous suspension of the wettable powder, which contains 50% 2:3-dichloro-1:4-naphthaquinone. An equal weight of Epsom salts (magnesium sulphate) was added before spraying.

"SR.406" (N-trichloromethylthio-tetrahydrophthalimide), as an aqueous suspension of a 50% wettable powder ("TP.13").

"J.49" (1-(4-chlorophenyl)-3:5-dimethyl-4-nitrosopyrazole), as a suspension of the pure material in 2% methyl cellulose. The amounts used were:—

J.49 $\frac{1}{2}$ -oz.

2% methyl cellulose .. $\frac{1}{4}$ -pint

Just before spraying, the mixture was diluted to 12 $\frac{1}{2}$ gallons.

Weather.

The contrast between the weather of the two summers is illustrated by Table 1, giving the records of rainfall, sunshine and mean temperature for each month from April to September in 1949 and in 1950.

TABLE I.
WEATHER RECORDS.

	Rainfall (in.)		Sunshine (hr.)		Mean temp. (°F.)	
	1949	1950	1949	1950	1949	1950
April	2.25	2.56	166.4	159.5	50.6	47.1
May	3.48	2.44	231.3	190.9	52.5	53.3
June	0.51	2.46	282.7	242.3	60.3	61.1
July	1.42	4.15	273.8	183.0	64.6	60.5
August ..	2.41	7.45	216.9	178.5	63.1	60.1
September ..	2.52	5.57	124.3	107.0	61.5	56.2
	<u>12.59</u>	<u>24.63</u>	<u>1295.4</u>	<u>1061.2</u>		

It is seen that in 1950 the summer rainfall was almost double that of 1949, while the hours of sunshine and mean temperatures were considerably lower.

Experimental.

Spraying methods. The major applications were made using a mobile power sprayer and spray brooms (pressure 400 lb./sq. in.: discs 6/64 in. aperture). For small groups of trees bucket pumps were used, special care being taken to ensure thorough application.

1949 Trials : Apples.

The 1949 trials were designed (1) to compare the effects of using glyoxalidine 341-C at the standard rate (1 qt./100 gal.) and at half this concentration (1 pt./100 gal.); (2) to test a new formulation, 341-SC, which was claimed to be free from the more phytotoxic unsaturated heptadecenyl derivative.

The programme as originally planned had to be modified owing to the effect of a shipping strike in delaying the arrival of the fungicides. All plots were therefore given a 2% lime sulphur pre-blossom spray, and the glyoxalidine sprays were then applied post-blossom on 30th May and 20th June, in comparison with 1% lime sulphur and with the unsprayed controls.

In the dry summer, scab development was negligible. No leaf infection estimates were made and when fruit scab was recorded on 20th September, the unsprayed fruits showed only 0.035% area scabbed. In the circumstances, no conclusions of any value could be drawn on the fungicidal performance of the sprays in this trial.

It was however possible to demonstrate significant differences in the phytotoxic effects of the various treatments. The results of the fruit yield counts made on 9th August, 1949, on Stirling, and on 20th September on Cox are summarised in Table II.

TABLE II.
APPLES : MEAN FRUIT COUNTS PER TREE, 1949.

Post-blossom Treatment	Variety	
	Cox	Stirling
Control	416.5	292.5
341-C 1 pt./100 gal.	410.3	194.0
341-C 1 qt./100 gal.	205.7	29.0
341-SC 1 qt./100 gal.	196.2	97.5
Lime-sulphur 1%	198.3	6.0

With the exception of the trees sprayed with the half-strength glyoxalidine 341-C, all the treated Cox trees show significantly lower yields than the controls. The figures for Stirlings are from 2-tree plots only, but indicate the very damaging effect of lime-sulphur and the less drastic, but still considerable phytotoxicity of the glyoxalidines in the 1949 season.

1949 Trials : Pears.

As in the apple plots, all the pears, except the control trees, received a pre-blossom 2% lime-sulphur spray. In the post-blossom treatments, given on 12th May and 8th June, 1% lime-sulphur was compared with glyoxalidine 341-C at 1 qt./100 gal. in four randomised blocks.

Scab attack on Williams was sufficient to justify a recording on 29th August, when fruits were classified into two categories only : clean and scabbed. Phytotoxicity on Comice was assessed on 13th October when the numbers and weights of fruits per tree were recorded. Both sets of figures are summarised in Table III.

These records indicate that in a season when post-blossom lime-sulphur sprays caused the shedding of practically the whole crop from the Comice trees, the glyoxalidine treatment was non-injurious. In scab control on Williams the glyoxalidine spray was superior to the lime-sulphur.

TABLE III.
PEARS : SCAB ASSESSMENT (WILLIAMS),
AND PHYTOTOXICITY RECORD (COMICE), 1949.

Treatment	Mean % scabbed fruit (Williams)	Mean no. fruit per tree (Comice)	Mean wt. (lb.) of fruit per tree (Comice)
Control	56.2	37.7	15
Lime-sulphur 1%	64.8	1.3	0.5
341-C 1 qt./100 gal.	31.7	41.1	16

1950 Trials : Apples.

Trial with glyoxalidine

The main 1950 trial on apples was made on a block of 95 trees in plot 23, comparing glyoxalidine 341-C at 1 qt./100 gal., pre-blossom and post-blossom, with lime-sulphur at 2% pre-blossom and 1% post-blossom. The sprays were applied on 27th April and 9th June.

Scab developed vigorously on the trees of Cox and an assessment was carried out on 18th September on the total crop of the 30 trees of this variety included in the trial. The results are shown in Table IV.

TABLE IV.
APPLES : SCAB ASSESSMENT ON FRUITS OF COX, 1950.

Treatment	Mean percentage fruit area scabbed
Lime-sulphur 2% and 1%	0.13
Glyoxalidine 341-C 1 qt./100 gal.	0.09
Control	0.54

The results for the two treatments are significantly different from those of the control trees, but not from each other.

Phytotoxic effects were compared by computing the mean numbers of fruit following the treatments on three varieties, Cox, Lane and Stirling. The results are assembled in Table V.

TABLE V.
APPLES : PHYTOTOXICITY RECORD.
MEAN NUMBERS OF FRUIT PER TREE FOLLOWING FUNGICIDE TREATMENTS, 1950.

Treatment	Variety		
	Cox	Lane	Stirling
Lime-sulphur	99	3.5	8.0
Glyoxalidine 341-C 1 qt./100 gal.	132	36.8	191.5
Control	148	51.5	388.5

Table V illustrates the well-known damaging effect of lime-sulphur on Lane and Stirling : comparison with Table II shows that glyoxalidine was relatively much less phytotoxic in 1950. On Cox, the yields on the glyoxalidine-sprayed trees were not significantly different from those on the controls, but the crop on the trees sprayed with lime-sulphur was significantly smaller.

A count was also made of russetting in these Cox fruits, the results being : lime-sulphur, 8.4% ; glyoxalidine, 6% ; control, 5.4%. The first figure is significantly different from the others.

Trials with other new fungicides

A preliminary field assessment of the fungicides listed below was made on Plot 23 by testing each material on four trees of Worcester Pearmain chosen at random. The following materials were applied on 28th April and 18th May :

Lime-sulphur, 2% pre-blossom and 1% post-blossom.

Phygon "XL" 0.05% (with 0.05% magnesium sulphate).

"TP 13" 0.2% (i.e. 0.1% trichloromethylthio-tetrahydrophthalimide).

Also on 9th June, a single application was made using 0.025% "J.49" (1-(4-chlorophenyl)-3:5-dimethyl-4-nitrosopyrazole).

Leaf recording of apple scab on 1st August and fruit recording on 23rd August gave the results arranged in Table VI.

TABLE VI.

APPLES : SCAB ASSESSMENT ON LEAVES AND FRUITS OF WORCESTER AFTER TREATMENT WITH CERTAIN NEW FUNGICIDES, 1950.

Treatment	Mean percentage area scabbed	
	Leaf	Fruit
Phthalimide (TP.13)	0.03	0.010
Lime-sulphur	0.10	0.023
Phygon XL	0.25	0.057
Pyrazole (J.49)	0.48	0.132
Control	1.30	0.258

Statistical analysis of the leaf results (excluding those from the single application of "J.49") shows "TP.13" > Lime-sulphur = Phygon > Control. The performance of the phthalimide in reducing leaf scab to less than 2½% of that on the control trees is noteworthy.

The fruit results were obtained from such a light crop that the figures did not justify statistical analysis.

"T.P.13" was tested for toxicity on trees of Stirling in the late summer. Four weeks after the application the percentage foliage retention was still 90 in the phthalimide-sprayed trees as compared with 38 in corresponding trees treated with a 1% lime-sulphur spray.

1950 Trials : Pears.

On the block of trees in plot 8 used in 1949, the standard glyoxalidine treatment was compared with unsprayed controls in 1950. The spray applications were made on 30th March and 9th June. A heavy attack of scab

developed, particularly on the fruit, and records taken 31st August from the crop of Williams showed a mean scabbed area of 1.86% on the glyoxalidine-sprayed pears as compared with 3.96% on the controls. On the Comice trees included in the trial, the crop was a light one, and the mean number of fruits per tree at harvest was 16.1 for the glyoxalidine treatment, 19.3 for the controls. These yield figures were not significantly different.

Discussion.

In the apple spraying trials, the results showed sharp contrasts between the two seasons. In 1949, the dry, hot weather virtually eliminated scab infection and made it impossible to compare the relative fungicidal values of the spray materials. On the other hand, the season was very favourable for the development of spray damage. It is not certain that the crop reductions on the trees sprayed post-blossom with the 341-C and 341-SC preparations in 1949 could be exclusively ascribed to these glyoxalidine sprays, since all the experimental plots had received in addition a 2% pre-blossom lime-sulphur application. However, Table II shows that, when all other circumstances were equal, the post-blossom spray with glyoxalidine 341-C at 1 qt. per 100 gal. caused a much greater crop reduction than the same spray used at half strength. This occurred on both Cox and Stirling, but on the latter a post-blossom 1% lime-sulphur spray was even more damaging than the full-strength glyoxalidine. There is an indication on the Stirling trees, but not on the Cox, that the 341-SC formulation was less damaging than the 341-C.

The possible relation of glyoxalidine damage to temperature has been explored in the U.S.A. by McCallan (6). His greenhouse trials showed that injury by glyoxalidines to apple foliage increased rapidly at high temperatures and became marked at 90°F. It is therefore possible that the degree of injury noted in 1949 would be unusual under English conditions, and even in that exceptional summer it did not exceed the damage induced by the standard post-blossom lime-sulphur spray.

In the cool, wet summer of 1950, when a programme of pre- and post-blossom glyoxalidine applications was compared with the standard lime-sulphur programme, no phytotoxic effects of the glyoxalidine sprays were recorded on Cox, and their phytotoxicity to Lane and Stirling was very considerably less than that of lime-sulphur. In this year, a satisfactory assessment of scab control was possible, and it was shown that one pre-blossom and one post-blossom spray with glyoxalidine 341-C at 1 qt. per 100 gal. reduced the amount of scab infection on Cox fruits to one-sixth of that on the controls. This equalled the fungicidal performance of lime-sulphur applied at 2% pre-blossom and 1% post-blossom, and confirmed the results obtained on Worcester Pearmain apples in 1948 (4).

On pears, the records of the fungicidal performance of glyoxalidine sprays are still meagre, and the counts on the fruits of Williams' Bon Chrétien indicated only a fair degree of control in both 1949 and 1950. The phytotoxicity trials on Doyenné du Comice have however given striking results, and indicate that the almost complete loss of crop which results from treating this variety with lime-sulphur can be avoided by substituting glyoxalidine applications.

The results with the other fungicides tested in the field against apple scab are preliminary, and more extensive trials will be needed before their performance can be satisfactorily assessed. The fungicidal efficiency of the phthalimide spray (SR-406), combined with its apparent safety on the sulphur-sensitive Stirling Castle, suggests that this material is of special promise.

Acknowledgments.

The help of Mr. S. H. Crowdy, Dr. R. S. Willison and Mr. A. H. Fielding in carrying out these trials is gratefully acknowledged.

Summary.

In 1949, in a hot, dry summer, apple scab development was too slight to compare fungitoxicity in the field trials at Long Ashton. A programme of 2% lime-sulphur pre-blossom, followed by two post-blossom glyoxalidine sprays at 1 qt./100 gal. was as phytotoxic to Cox as the standard lime-sulphur programme but less phytotoxic to Stirling.

In the cool, wet season of 1950, a programme of one pre-blossom and one post-blossom spray with glyoxalidine 341-C at 1 qt./100 gal. equalled the standard lime-sulphur programme in scab control on Cox's Orange Pippin apples and was less phytotoxic. On the varieties Lane's Prince Albert and Stirling Castle the glyoxalidine sprays were much less phytotoxic than lime sulphur.

Glyoxalidine spraying of the pear variety Doyenné du Comice, which is seriously damaged by lime-sulphur, caused no decrease in yield in 1949 or 1950.

Results are given of preliminary apple spraying trials with the naphthaquinone spray Phygon "XL", a substituted pyrazole ("J.49") and a phthalimide preparation (SR-406). The last-named was very promising in its fungicidal performance and freedom from phytotoxicity.

REFERENCES.

- (1) BYRDE, R. J. W. (1950). Experiments on the control of brown rot of fruits: progress report, 1948-49. *Long Ashton 1949 Ann. Rep.*, 81-89.
- (2) KIRBY, A. H. M. (1950). Plant protective chemistry. *East Malling 1949 Ann. Rep.*, 45-47.
- (3) MARSH, R. W. (1947). Fruit spraying trials with certain recently-introduced fungicides. *J. Pomol. hort. Sci.*, **23**, 185-205.
- (4) MARSH, R. W. (1948). A supplementary note on fruit spraying with a glyoxalidine preparation in 1948. *J. hort. Sci.*, **24**, 284-287.
- (5) MARSH, R. W. (1950). Report on a visit to Canada and U.S.A. to study research and development in agricultural fungicides. *Agric. Res. Coun. Rep. No. 12241*, pp. 1-36.
- (6) MCCALLAN, S. E. A. (1950). Factors influencing spray injury by glyoxalidine derivatives to potted apple trees. *Contr. Boyce Thompson Inst.*, **16**, 21-26.

AN EXPERIMENTAL "AIR FLOW" SMALL VOLUME SPRAYER AND DUSTER. ABRIDGED SPECIFICATION.

By H. G. H. KEARNS and N. G. MORGAN.

The most widely used method of application of insecticides, fungicides and weed killers is by spraying diluted materials in large volumes of water. This procedure has two major advantages, firstly the target cannot be over-sprayed as the excess liquid runs off and thereby automatically reduces the risk of phytocidal damage; secondly it provides a better chance of obtaining adequate coverage. The mechanical obstruction presented to the passage of droplets by foliage is considerable and increases rapidly with the height and density of the target. The smaller the size of the droplets the greater the difficulty of securing coverage, indeed some assemblages of vegetation will filter out every droplet on the outside and thereby create a zone of extremely high concentration. The normal method of large volume "run off" spraying entails not only the use of large quantities of water but a very high waste of the spray material. These losses are greatest on tree crops and smallest on shallow ground crops. The recovery figures, i.e., the toxicant usefully retained by the crop may be as low as 5 per cent. when trees are sprayed in winter, yet this loss is accepted as economic.

The application of small volumes of concentrated materials on crops has attracted attention for many years. The difficulties of finding non-phytotoxic yet highly toxic materials and of designing efficient machinery has limited the progress of this method. The recent development of highly toxic organic insecticides, such as D.D.T. and B.H.C., and of hormone weed killers has stimulated development of machines, but safe fungicides for many crops by this method of application have yet to be found.

There are some problems which appear particularly favourable for the successful application of small droplets of concentrates, for example locusts on the ground where they are a relatively easy target when compared to small insects feeding amongst foliage on trees. Small volumes of highly concentrated materials are fairly readily applied to such ground crops as grass, cereals, potatoes, etc., by the simple procedure of overriding the crop and spraying from a "boom" by means of hydraulic pressure. These crops are well suited to this method of spraying on calm days as the flight of the small droplets to the target is short and in a downward direction. "Boom" spraying has the disadvantage that it is necessary to traverse the crop every 20-35 ft. with a tractor-drawn or self-propelled machine.

If the "boom" is erected vertically in the form of a "mast" the ultimate goal of the small droplets is very much in the hands of the prevailing wind. The small amount of energy in the droplets limits their distribution on foliage and where there is any quantity of foliage practically no coverage in depth occurs. It is for this reason that this method will only provide some measure of reasonable uniformity of coverage on open ground or a closely mown grass surface.

The efficiency of small volume spraying depends on the method of conveying the droplets to the target. It is not possible to increase to any appreciable extent the length of flight of the small droplets ejected from a nozzle by increasing the hydraulic pressure.

The most simple method of distributing small droplets is by means of

moving air produced by a large fan. Several such "air flow" mist machines, mostly American, have been designed but little data is available on their specification or performance.

An experimental machine was built at the Research Station to ascertain the size and type of fan required for "air flow" mist spraying of crops. It was found in experiments with an air volume of 2,000 cu. ft. that little was gained by high pressures, thus 4 lbs. per sq. in. pressure requiring about 60 B.H.P. gave a most disappointing performance. It was evident that a large air volume was desirable and that pressures in excess of 12 in. W.G. pressure were not necessary.

It appeared that a fan with a delivery of 4,000–5,000 cu. ft. at 4" W.G. pressure was the minimum size likely to be suitable for "air flow" drift spraying. Data was not available on the importance of volume and pressure when using volumes greater than 4,000 cu. ft. Data on the behaviour of droplets of plant protection materials on a wide range of crops was urgently required. An experimental general purpose machine has been designed so that a wide range of performances can be studied. The fan is capable of delivering up to 20,000 cu. ft. of air at 5" W.G. pressure and 10,000 cu. ft. at 12" W.G. pressure. The volume of spray liquid sprayed can be adjusted from a few fluid ozs. to 10 gallons per minute at up to 150 lbs. per sq. in. pressure. The air stream can be delivered in any direction.

The machine described and illustrated is a modified model specially designed for experimental work on the control of locusts. This machine can be used for the application of solutions, suspensions or dusts. Although it is shown (Fig. 1) mounted on a tractor-trailer it is designed for mounting on the platform bed of a lorry.

Broad Specification.

The machine comprises the following principal items :—

- I. Chassis.
- II. Engine unit with clutch, gear-box, radiator, fan drive, power-take-off auxiliary drive, instrument panel and controls.
- III. Fan.
- IV. Tanks, tank filters.
- V. Tank filler, agitator pump.
- VI. Suction filter, pressure pump, delivery return circuit to spray nozzles.
- VII. Fan nozzle and spray nozzle assemblies.
- VIII. Dusting equipment.

I. The Chassis.

The chassis is of welded construction of 3" × 2" channel made for ease of packing and shipment in two main sections and a sub-section. The two main sections are bolted together, total length 11 ft. 3 ins. On one of these the engine and ancillary drives are mounted, on the other half the fan, tanks and dusting equipment. The sub-section 1 ft. 9 in. long is a detachable extension of the chassis and acts as a support for the fan delivery trunking.

The chassis was bolted to a tractor-trailer frame from which the drawbar and complete axle could be readily removed so that the whole assembly could be installed on the bed of a lorry. This arrangement aimed at a stiff construction able to withstand the strains caused by very rough road and cross-country travelling.

II. *The Engine Unit.*

Comprises an industrial Ford V-8 engine, clutch and 4-speed gearbox. The unit was mounted on substantial support brackets at a height to facilitate easy hand cranking of the engine and easy drainage of the oil sump when the chassis was fitted to the lorry floor. The engine was mounted at the front on the two normal car engine rubber mountings and the gearbox in a circular flexible housing. This form of mounting gave vibrationless running at all speeds.

Cooling is by a specially built rubber mounted 4-gallon capacity radiator for tropical conditions with the radiator grill facing the back of the lorry cab. Air is drawn through the radiator by a six-bladed fan with a double vee belt drive. The engine has two water circulating pumps. The front of the radiator is fitted with a 24-mesh brass gauze grill to prevent blocking of the honeycomb by indrawn insects, seeds, etc. The radiator was fitted with sufficient clearance from the engine to permit of easy access to the ignition distributor and water circulating pumps. The engine is fitted with an electric self-starter, dynamo and accumulator and coil ignition. The exhaust fumes are picked up and discharged by the axial flow fan. The gearbox is fitted with a power-take-off drive unit with a shaft extension and control so that it can be placed in and out of gear: the speed of the shaft to that of the engine is in the ratio of 1:0.7. This unit provided the drive for the pressure and agitator pumps and for the dust feed gearbox.

The engine controls comprise an instrument panel, ignition switch, self-starter button, ammeter, oil pressure gauge, cooling water thermometer and throttle control. Clutch, gearbox and power-take-off control rods are grouped together in convenient positions.

The drive between the gearbox and fan comprises two needle-bearing type universal joints and a short splined shaft. The former allow angular movement between fan and engine units and the latter, one end of which is a sliding fit in one of the joints, allows axial movement and is shielded by two dust hoppers.

III. *The Fan.*

The fan is of the two stage axial flow type of 30 in. internal diameter with fully balanced aluminium alloy guide vanes and runners. The tip clearance of the blades is $1/16$ in. approximately. The runners are mounted on a 2 in. shaft running in two heavy duty self-aligning ball bearings which are surrounded by cowling to prevent interference with the air flow, and the bearing on the delivery end of the fan is fitted with a large anti-turbulence cone deflector. The body of the fan is rolled plate reinforced by three angle hoops. The whole is a welded structure 39 in. long, weight 5 cwt.

Direct drive at engine speed was chosen as it provided the most reliable lay-out and avoided all the complication likely to be met from a multi-rope drive under African conditions. The fan is designed for continuous operation at 2,500 r.p.m., and at its maximum W.G. pressure requires about 31 B.H.P. and the auxiliary drives about 6 B.H.P., a total of 37 B.H.P. The engine at 2,500 r.p.m. develops about 50 B.H.P. so it is in considerable excess of requirements even after allowing for a drop in efficiency after considerable use. This apparent waste of developed power does not cause any appreciable increase in fuel or oil consumption.

IV. *Tanks (Plate IX, Fig. 2).*

A 60-gallon rectangular spray tank with a rounded bottom is fitted on each side of the axial flow fan. Each tank, 38 in. long $\times$ 41 in. high $\times$ 12 in.

wide, is fitted with a non-spill type $7\frac{1}{2}$ in. diameter filler hole and bayonet type of cover lid attached by a chain. Each filler hole contains a cylindrical 30 in. $\times$ 7 in. filter basket made of perforated plate with $3/16$ in. holes, one being lined with a 30 in. mesh phosphor bronze gauze sleeve. Each tank is fitted with a non-spill dip stick graduated in 5 gallons. The tanks were hot galvanised finish after manufacture. The two tanks are inter-connected by a $1\frac{1}{2}$ in. bore pipe and either one or both tanks can be connected by means of valves to the agitator and filler pump. Each tank can be completely drained for cleaning purposes by a full-way valve.

A 10-gallon cylindrical petrol tank is fitted on top of the fan between the spray tanks, which protect it from damage. This tank is filled by a hand operated rotary pump and the level checked by a gauge on the instrument panel.

V. *Tank Filler and Agitator Pump (Plate XI, Figs. 3 and 4).*

A $1\frac{1}{2}$ in. bore self-priming centrifugal pump fitted with oil resistant packings and valve seats is driven from the power-take-off drive by means of 2 of $\frac{1}{2}$ in. split link belts. The suction and delivery ports are fitted with oil resistant hose connections to permit of movement of the pump for adjustment of the drive belt tension and easy servicing. At an engine speed of 2,500 r.p.m. the pump is driven at 3,500 r.p.m. and it fills or agitates the contents of the tanks at the rate of 30–40 g.p.m.

The suction circuit for agitation is connected to the bottom of each tank and controlled by a fullway valve, and the liquid before entering the pump passes through a large 8 in. $\times$ 4 in. filter with an easily removed and cleaned element of 30 mesh phosphor bronze gauze. When the pump is used for filling the tanks, a blank cap is removed from the suction circuit and a suction hose attached. In this case the fluid does not pass through the suction filter.

The oil resistant $1\frac{1}{2}$ in. suction hose is fitted one end with a full-way valve and lug nut for attachment to the suction circuit, and the other end is fitted by means of a lug nut to a barrel suction pipe (Fig. 5). Two lengths of 20 ft. suction hose which can be connected together are provided. The barrel pipe is fitted with an air vent valve so that spilling can be reduced when the pipe is removed from the barrel, and the "full-way" valve on the other end of the hose prevents the contents of the tube spilling over the operator when it is detached from the machine.

The delivery circuit is arranged so that the fluid flows *via* a control valve into the filter basket of either spray tank. It also supplies by means of a bypass the high pressure gear pump. This arrangement ensures priming and it also reduces the risk of the pump running dry and seizing. If required the centrifugal pump can be used for pumping liquid from one outside container to another. This is carried out by closing the tank circuit and fitting suction and delivery hoses to the lug nut adaptors on the pump circuit. The pump is normally used for the agitation of the spray liquid and this may be controlled or stopped by means of the delivery valves into the tanks.

VI. *The High Pressure Pump Circuit (Plate IX, Fig. 4).*

The spray fluid is fed to the spray nozzle assembly by means of a $\frac{3}{4}$ in. B.S.P. gear pump with a maximum delivery of 10 gallons per minute at 100 lbs. per sq. in. at 1,000 r.p.m. It is run at 800 r.p.m. at engine speed of 2,500 r.p.m. and the drive is by means of two $\frac{1}{2}$ in. split link vee belts on a $8\frac{1}{2}$ in. O.D. pulley driven from a 2 in. O.D. pulley on the shaft of the centrifugal pump. The pump is made of phosphor bronze with a stainless steel shaft with an adjustable braided hemp graphite packing. A relief valve which can be set to

release at any pressure from 50 150 lbs. per sq. in. is incorporated in the design. Short lengths of $\frac{1}{2}$ in. bore oil resistant high pressure flexible hoses are fitted to the suction and delivery between the pump and the plumbing for ease of removal and adjustment of the belts. The pump is fitted with a pressure gauge protected by a wheel valve. The first section of the delivery circuit is a short length of oil resistant rubber hose attachment to a trigger tap which can be kept open or instantly closed by a lever and ratchet. This tap is fitted to $\frac{3}{4}$ in. bore steel tubing which incorporates three accessible easily cleaned filters arranged in line. The first filter is fitted with an element of 40 mesh gauze; the second with 50 mesh; and the third with 60 mesh. The aperture of mesh is selected according to the type and aperture of the spray nozzles. The fluid after passing through the filters is fed through oil resistant hose to a manifold with three outlets each controlled by a $\frac{3}{8}$ in. B.S.P. valve. A second similar manifold is fitted and this is attached by means of rubber hose to a return circuit pipe which discharges through a relief valve into one of the spray tanks. The return circuit is of value when using suspensions as it avoids the difficulties resulting from settling out of solid matter in the delivery circuit; the relief valve on the return circuit is set to release at a lower pressure than that on the pump. The nozzle assembly is attached by hose to the manifolds.

Fan Nozzle Assembly (Plate X, Fig. 6).

A cylindrical tube approximately 2 feet long is attached to the delivery end of the fan. It tapers down from a diameter of 30 in. to 24 in., and the last 8 in. length is straight. To this tube is attached a swivel joint supported on large rollers (Fig. 6) and operated by a capstan handle. A telescopic bend is attached to the swivel base. A 3-foot tapered nozzle made up of three detachable sections of equal length is fitted to the telescopic bend. The nozzle can be used with apertures of 1 ft., 8 in., 1 ft. 5 in., 1 ft. 2 in., and 11 in. O.D. (Fig. 7). The swivel base provides a movement of 360° and the telescopic bend of 45° . The inside of the telescopic bend is fitted with guide vanes. Provision is made for locking the swivel and bend in any required position. Owing to the size and weight of the assembly it can only be moved easily by means of a long tubular bar when the fan is running at its normal speed.

The Spray Nozzle Assembly (Plate X, Fig. 8).

The air nozzle was designed so that alternative methods of "atomising" the spray fluid could be investigated. The 11 in. aperture allows full advantage to be taken of the maximum air velocity of 150 m.p.h. This was ample for the formation of droplets from liquid fed through simple apertures.

However, for experimental purposes three rings of swirl type nozzles are fitted in the air stream on the periphery of the 20 in. air nozzle. The rings are 22 in. diameter and made of $\frac{3}{4}$ in. copper tubing, each with 24 equally spaced $\frac{1}{4}$ in. B.S.P. (1) nozzles fitted to brass saddles. The rings are 4 in. apart, the saddles on the inner ring being fitted with $3\frac{1}{2}$ in. long extension tubes, and the middle ring with $1\frac{1}{2}$ in. tubes. This arrangement brings the nozzle into the air stream whatever the position of the air nozzle and avoids any mechanical obstructions in the path of the spray droplets.

The droplet size and throughput is adjusted by the size of disc aperture and type of swirl plate in the nozzles. If all the nozzles are used simultaneously the throughput is adjusted to suit the capacity of the pump. The normal nozzle assembly is $\frac{2}{64}$ th discs and "small volume" swirl plates spraying at a flow pressure of 75-100 lbs. per sq. in.

PLATE IX.



Fig. 1.



Fig. 2.

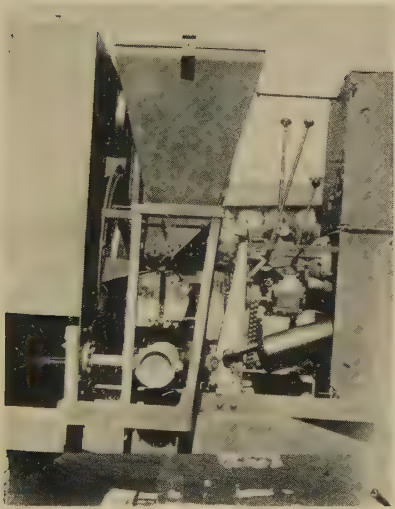


Fig. 3.

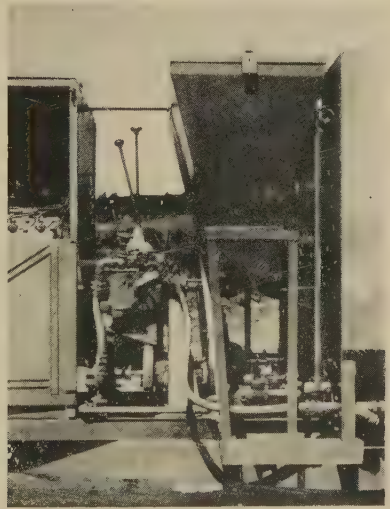


Fig. 4.

Fig. 1. "Air Flow" small volume sprayer and duster mounted on tractor trailer chassis.

Fig. 2. Top view showing from left to right two spray tanks, two dust hoppers and the engine unit.

Fig. 3. Offside view showing dust feed-box, venturi trough leading into the fan, suction filter, general arrangement of the main controls, instrument panel and power-take-off drive.

Fig. 4. Nearside view showing the general arrangement of the circuits of the centrifugal and gear pumps.

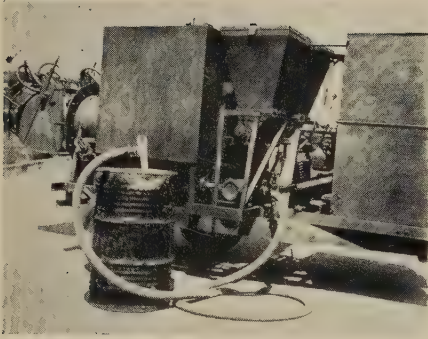


Fig. 5.



Fig. 6.

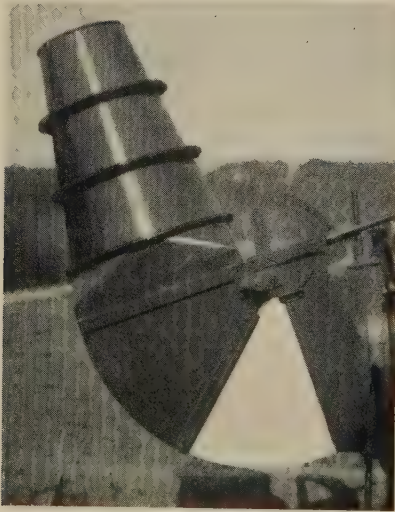


Fig. 7.



Fig. 8.

Fig. 5. Showing method of filling spray tanks by means of a barrel pipe and suction hose fitted to the centrifugal pump.

Fig. 6. Fan nozzle assembly showing capstan roller mounting, telescopic bend with 1 ft. 8 in. aperture, triple ring spray nozzle assembly and petrol filler pump.

Fig. 7. Fan nozzle extension piece for experiments with high velocity air.

Fig. 8. Rear view of fan nozzle showing air guide vanes inside telescopic bend and delivery and return hose lines to the spray nozzle rings.

One or more nozzle rings are used according to experimental requirements..

Dusting Equipment (Plate IX, Fig. 3).

The machine may be used for the application of suitably compounded dusts. The dust is fed into the suction side of the fan and delivered by the air nozzle. The smaller the aperture of the nozzle the further the dust can be projected into the air. The inert filler of the dust must be of a non-abrasive type such as Kaolin to avoid serious wear of the tips of the aluminium alloy blades. Such wear would not only reduce the efficiency of the fan but any uneven wear would upset the balance and cause breakdown of the runner.

The dusting equipment comprises two similar self-contained units installed between the fan and engine on a welded chassis. The whole assembly can be readily removed from the machine. Each unit consists of a dust hopper, a dust feed box and a venturi trough.

The hopper is a steep walled mild steel container of 2 cwt. capacity with a water-tight cover. The hopper is bolted to a robust feed box made up of welded plate. The feed box contains a rotating spindle with three rows of pegs. The spindle of each dust unit is connected to a reduction gear-box which is driven through a chain drive from the power-take-off shaft of the engine. The dust is fed by the rotating spindle to a long slot from which it is removed by the suction of the fan through the venturi trough. The width of the slot can be adjusted and the amount of dust applied to the fan determined. A quick acting control is fitted so that the dust flow can be opened or shut off at once. Both dust units are operated at approximately the same flow rate at the same time in order to avoid disturbing the balance of the fan. During dusting an earthing chain should be fitted to the chassis of the machine to avoid build up of electrostatic charges.

Acknowledgment.

A grant from the Agricultural Improvement Council is gratefully acknowledged as it enabled much of the experimental work to be developed to a practical stage.

REFERENCE.

- (1) KEARNS, H. G. H. and MORGAN, N. G. A lightweight spray nozzle ($\frac{1}{4}$ in. B.S.P.) for "small" and "large" volume spraying. *Ann. Rept. Long Ashton Res. Sta.*, 1949, pp. 111-114.

A GENERAL PURPOSE WHEELED MANUAL SPRAYER.

By H. G. H. KEARNS and N. G. MORGAN.

There is an ever increasing need in many parts of the Colonies and elsewhere for a simple, reliable, easily moved and manually operated spraying machine. It is required to replace or augment the pneumatic knapsack. Small power operated machines are none too successful owing to the difficulty of operation and maintenance by unskilled native labour and lack of facilities for repairs.

The machine is required for a wide range of duties including the spraying of buildings and huts with insecticides for the control of mosquitoes. In addition to these uses the machine is most convenient for carrying out small spray trials on ground and small bush crops as it is easy to operate and clean.

Broad Specification.

A machine was designed with a tank of 12-gallon capacity carried in a light but strong rubber-tyred wheeled chassis. A pump was fitted within the tank which was fitted on trunnion bearings so that it remained level whatever the gradient of the ground and thereby reduced loss of liquid from slopping. The pump can be operated with the tank in the chassis or the tank can be instantly and with little effort parked on the ground or the tank can be carried on a pole by bearers over a difficult terrain.

Two types of pump can be fitted in the tank ; (a) a stirrup pump or (b) a 2-plunger beam pump. Both are fitted with an agitator capable of maintaining dispersible powders in suspension. The performance of the pumps depends on the number of strokes per minute : the beam pump is more convenient and easy to operate but it is much more expensive than the stirrup pump. The working pressure is easily maintained at 75 lbs. per sq. in. A readily cleaned and renewable filter is fitted to prevent blockage of nozzle disc apertures.

The lance equipment includes nozzles (1) suitable for small or large volume spraying and oil resistant light weight hoses. Either pump can be operated with two lances when "small" volume spraying is carried out. The machine requires one operator for the pump and one for each lance. The performance is considerably superior to that obtained from two of the best type of pneumatic knapsack sprayers both in quality of spraying and saving of time. The weight of the complete machine, less lances and hoses, is for the stirrup pump model, 86 lbs. approx., and for the beam pump model, 96 lbs. approx.

Specification L.A.R.S., No. 256 and 257.

Chassis and Tank (Plate XI, Fig. 1).

The chassis (Plate XI, Fig. 2) is of simple rigid and welded construction of 1" O.D. $\times$ 14 G. ($\frac{3}{4}$ " conduit) steel tubing with 1 $\frac{1}{4}$ " O.D. (1" conduit) axle tube. The two side members are of triangular shape held together by three transverse members, one acting as the handle and another as an axle tube. A 1" axle is fitted through the axle tube and carries two 14" $\times$ 3" steel disc wheels with cushion rubber tyres on plain bearings. Four vertical brackets on which the hose is reeled are welded on top of the chassis and clips are fixed

on the side members to carry the lance or lances. Weight of chassis and wheels, 56 lbs. approx., and wheels only 35 lbs. Overall width of machine, 2' 4½"; length in parked position, 3' 9". The chassis is finished signal red in oil resistant paint.

The cylindrical tank, 15" diameter $\times$ 15" deep, is of 22-gauge mild steel with the top edge wired and a force fit flat band fitted around the bottom. Capacity 12-gallons approx. Two of 2" $\times$ ¼" $\times$ ⅛" channel sections to act as pump supports and two 20" long $\times$ 2" $\times$ ¼" $\times$ ⅛" channel sections to act as foot rests and to reinforce these supports are welded to the bottom of the tank. Trunnion brackets and pole carrying handles are welded to the top of the tank. A push-in spring-fit cover lid with an aperture for a beam pump operating lever or a stirrup pump is provided. The tank is supported but free to move on two trunnions welded to the chassis tubing so that the tank remains level. It can be immediately released from or attached to the chassis by a simple movement of the handle controlled by foot pressure on the axle (Plate XI, Fig. 2).

When a stirrup pump is used the tank is fitted with a galvanised light channel section cross support. The upper portion of the pump barrel is firmly clamped to the support and the foot filter is located on the bottom of the tank between three vertical steel pegs which are welded to a plate bolted or welded to the tank. When a beam pump is used it is bolted to the reinforced tank bottom. The lever when not required is conveniently stowed inside one of the chassis side members and retained by a bayonet fitting and drop pin. A combined filter and hose connection is fitted into the top portion of the tank.

The tank, cover lid and all fittings are hot galvanised after manufacture. The approximate weight of the tank is 22 lbs.

Stirrup Pump (Plate XI, Fig. 4).

Any well designed double acting stirrup pump* will be satisfactory. The normal foot stirrup and tube clamp bracket is removed so that the barrel can be clamped to the cross bracket in the tank. The radius of the clamp and the spacing of the pegs on the bottom of the tank must suit the pump.

An agitator is fitted to the pump in the form of a perforated plate, so that the plate moves up and down with the pump plunger. A cylindrical tank of 18" diameter requires an agitator plate of approximately 80 sq. in. with ten 1" diameter holes.

The delivery outlet of the pump must be fitted with a combined filter and hose connection. The gauzes in the filter should be 40 mesh (S.W.G.) when spray nozzle disc apertures of 3/64th in. and greater are used and 60 mesh for smaller sizes.

The pump should be capable of delivering not less than 5 pints at 40 strokes per minute at 50–75 lbs. per sq. in. pressure. The weight of a stirrup pump is 3½–4 lbs. and the agitator 2 lbs. approximately.

A beam two-plunger pump (Plate XII, Fig. 5).

A beam pump comprises a basal casting with two plungers which are operated by moving a lever to and fro.

The body and air vessel.

The body and air vessel are two separate aluminium, brass or phosphor bronze castings, brass or phosphor bronze being necessary to avoid extensive

* Long Ashton Research Station Specification No. 302.

corrosion when copper containing spray fluids are used. Into the body two cylinders, two delivery suction valves with filter assemblies and two delivery valves are screwed. An oval section air vessel $5" \times 3\frac{1}{2}" \times 2"$ approximately is fitted over the delivery valves. The delivery outlet is sited near the bottom of the air vessel and to it is attached a brass relief valve which can be set to release at predetermined pressure. An oil resistant $\frac{1}{4}"$ bore hose or copper tube is fitted between the delivery and the filter-hose connector unit on the tank.

The cylinders.

The two vertical interchangeable cylinders of brass tube $1\frac{1}{4}"$ bore $\times$ 6" long (working stroke of 3") are brazed into base collars which screw into the main body.

The plungers.

Each plunger consists of a double cup assembly mounted on a brass piston. The cups are of leather and impregnated with colloidal graphited oil. The whole assembly is fitted to a connecting rod with a gudgeon pin.

The valves.

Each plunger has a suction and delivery valve.

The valve cages, seats and balls of the suction and delivery valves are interchangeable. The valve seats are of reversible type and of phosphor bronze with $\frac{3}{4}"$ stainless steel or phosphor bronze balls. The suction valves form part of the foot filter assembly and are screwed into the body casting. The delivery valves are sited within the air vessel. The valve cages screw down on to the seats.

The rocker beam and connecting rods.

The rocker beam is mounted on the top of the air vessel and to it are attached the pump lever and two connecting rods of the plungers. The lever is 3' long $\times$ $\frac{5}{8}"$ diameter and made of steel tubing and mounted in the centre of the beam.

The suction strainers.

Detachable circular strainer bodies are screwed to each suction valve assembly. Each is covered with monel metal gauze of 40 mesh of 9 sq. in. approx. which can be readily replaced.

The agitator.

A single vertical metal paddle $10" \times 3\frac{3}{4}"$ is attached to the pump beam and moves to and fro in the tank as the pump lever is operated.

Attachment to the tank.

The pump casting is bolted with four brass bolts to the bottom of the tank: each of the bolts is made leak proof by means of fibre washers and self-setting liquid packing. The channel members under the tank take the strain when the pump is operated.

Performance.

The delivery of the 2-plunger beam pump at 45 strokes per minute at 75 lbs. per sq. in. is approximately three pints. The pump is capable of operating up to 200 lbs. per sq. in. pressure, but this is excessive and likely to burst the hoses, so the relief valve is set at 80 lbs. per sq. in. The relief valve also allows a rapid stroke rate when required for increased agitation of the spray liquid in the tank without pressure increase in the hoses.

Lance* and Hose Equipment.

- (1) $2 \times 30' \frac{1}{4}"$ bore oil resistant high pressure hose (working pressure 150–200 lbs. per sq. in. Each hose with light pattern $1" \text{ W.}$ or $\frac{3}{4}"$ B.S.P. lug nuts, jubilee hose clips.
- (2) $1" \text{ W.M.} \times 1" \text{ W.M.}$ or $\frac{3}{4}" \times \frac{3}{4}"$ B.S.P.M. hex. hose to hose connector.
- (3) $1-18"- \frac{3}{4}"$ lance, one end $\frac{1}{4}"$ B.S.P.F. (for item 7), one end $\frac{1}{2}"$ B.S.P. loose lug nut (to fit item 9).
- (4) 1–Extension telescopic lance ($30" \times \frac{3}{8}"$ O.D. straight tube in $30" \times \frac{1}{2}"$ O.D. tube 18 G.), $\frac{3}{8}"$ B.S.P. stuffing gland. $\frac{3}{8}"$ tube with $\frac{1}{4}"$ B.S.P.F. end. $\frac{1}{2}"$ tube with $\frac{1}{2}"$ B.S.P. loose lug nut to fit trigger tap (item 9).
- (5) 1–2-nozzle broom head, nozzle spacing $9"$ apart. $\frac{1}{4}"$ B.S.P.M. small volume nozzles. (Complete $1/32"$ disc and $1/64"$ swirl plate and filter). Each nozzle set in line with lance. Broom $5/16"-18 \text{ G.}$ tube. Nozzle sockets $\frac{1}{4}"$ B.S.P.F. and lance adaptor $\frac{1}{4}"$ B.S.P.M.
- (6) $1-60^\circ$ elbow $\frac{1}{4}"$ B.S.P.F. $\times \frac{1}{4}"$ B.S.P.M. to fit (4) and (3).
- (7) $1 \frac{1}{4}"$ B.S.P. nozzle (complete with $3/64"$ disc and 2-slot swirl plate and filter).
- (8) $1-\frac{1}{4}"$ B.S.P.M. small volume nozzle (complete $1/32"$ disc, $1/64"$ swirl plate, filter).
- (9) 1–Full-fist type trigger tap with $\frac{1}{2}"$ B.S.P.M. $\times 1" \text{ W.M.}$ or $\frac{3}{4}"$ B.S.P.M. hose connector.

* For particulars of the design of the nozzle see (1).

Spares.

- (10) $1-1" \text{ W.M.} \times 1" \text{ W.M.}$ or $\frac{3}{4}" \times \frac{3}{4}"$ B.S.P. hose connector.
- (11) $12-\frac{1}{2}"$ B.S.P.M. leather washers.
- (12) $24-1" \text{ W.}$ or $\frac{3}{4}"$ B.S.P. lug nut leather washers.
- (13) $4-\frac{1}{4}"$ hose jubilee hose clips.
- (14) $2-\frac{1}{4}"$ hose mender tubes.
- (15) $2-\frac{1}{4}"$ B.S.P. nozzles (complete $1/32"$ disc, $1/64"$ swirl plate, filter).
- (16) $24-\frac{1}{4}"$ B.S.P. nozzle cap lead gaskets.
- (17) $12-\frac{1}{4}"$ B.S.P. nozzle discs $2/64"$ aperture.
- (18) $12-\frac{1}{4}"$ B.S.P. nozzle discs $3/64"$ aperture.
- (19) $12-\frac{1}{4}"$ B.S.P. nozzle discs $4/64"$ aperture.
- (20) $12-\frac{1}{4}"$ B.S.P. nozzle swirl chamber plates— $1 \times 1/64"$.
- (21) $12-\frac{1}{4}"$ B.S.P. nozzle swirl plates—2 side slot $\times 1/16" \times 3/32"$.
- (22) 24–Filters with apertures in gauze less than $1/32"$ (60 mesh).
- (23) 12–swirl chamber rings.
- (24) $24-\frac{3}{8}" \times 5/16" \times 1/32"$ fibre washers for $\frac{1}{4}"$ B.S.P. nozzle adaptors.
- (25) 12" graphite hemp packing for trigger tap gland nut.
- (26) 4–spare leather plunger buckets (impregnated with colloidal graphite).
- (27) $1-2\frac{1}{2}"$, 0–200 lbs. per sq. in. pressure gauge on adaptor to fit at base of lance for testing purposes only.
- (28) 1–2-way $1" \text{ W.M.}$ or $\frac{3}{4}"$ B.S.P.M. hose connector with loose $1" \text{ W.}$ or $\frac{3}{4}"$ B.S.P. loose lug nuts (to permit use of two lances).

Operation of the machine.

The machine is most conveniently operated with the chassis parked on the ground and the tank held in a fixed position by means of two steel eye pins. These pins pass through holes in the brackets on the chassis and base of the tank. When not in use the pins are fitted into the bracket holes and are chained to the chassis.

In the model fitted with the beam pump, one foot is placed on the handle cross member and the pump lever moved to and fro. The stirrup pump can be operated in the chassis but it may be found more convenient to operate on the ground.

If the tank is used apart from the chassis it is readily parked on the ground (Plate XI, *Fig. 3*). The tank in this event is held steady during the operation of the pump by placing one or two feet on the foot rests fitted to its base.

Two lances are provided and the choice depends on the nature of the target. The telescopic lance is convenient for reaching objectives high up from ground level. The length of the lance is easily adjusted by slackening the stuffing box nut and pulling out the inner lance tube to the required length. It is important to tighten up the gland nut before spraying. A single nozzle may be used or, if a wider area is to be sprayed, more especially with "small volumes," it is more convenient to use a two-nozzle broom head. The angle of the nozzle or broom head can be set to 45° or 60° by fitting an appropriate elbow piece.

A washer should be placed into the nozzle adaptor before the nozzle is lightly screwed home to make a leak-proof joint.

The performance of the nozzle (1) is determined by the size of the aperture in the disc and the type of swirl plate fitted. The "small volume" swirl plate has a shallow chamber and one side slot and it is suitable for the $2/64$ " discs. Discs with above $3/64$ " require a 2-side slot swirl plate. In the case of the "small volume" assembly the swirl plate is inserted first into the nozzle counter bore and then the disc. If $3/64$ " or greater disc apertures are used, a 2-side slot swirl plate is first inserted then a swirl plate ring and finally the disc. The whole is kept leak-proof by a hexagonal nozzle cap with a lead gasket fitted inside. When $3/64$ " discs and less are used it is desirable to fit a filter into the base of the nozzle. This filter is readily cleaned by washing over with a brush and it is only provided as an emergency and should it block frequently it is evident that the main filter on the pump is fitted with gauze of too large an aperture.

Maintenance.

The two types of pump require little attention. The ball valve assemblies should give very long service before requiring renewal. Occasionally after storage the suction ball will stick to its seat and generally it may be dislodged by tapping the pump sharply. The leather buckets on the plungers gradually lose their lubricant, more especially when using kerosene and fail to hold pressure. New buckets are readily fitted. If the pumps are stored for any period after use, it is desirable to oil the buckets with graphited oil. In the case of the stirrup pump this is readily done by pouring in a few drops of oil through the foot valve with the pump in an inverted position. The beam pump buckets may be lubricated by pouring in oil on top of the plungers. In the case of both pumps a few strokes will spread the oil. Lubrication of the pivot pins on the beam pump is by means of the spray fluid and in time wear will develop which can only be remedied by reaming out the bearings and yokes and fitting oversize pins.

The only point on the stirrup pump apart from the leather buckets that requires regular attention is the stuffing gland. This is packed with braided graphited hemp and as soon as a leak develops the gland nut should be screwed up by hand. In time this will fail to prevent leakage and as undue tightening should be avoided a new packing must be fitted.



Fig. 1. The complete spray machine at rest on the ground.

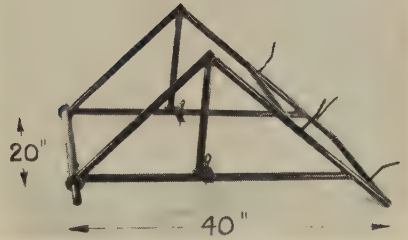


Fig. 2. The chassis, less wheels and tank, showing the principal dimensions.



Fig. 3. The tank being parked.



Fig. 4. The stirrup pump with agitator.



Fig. 5. The beam pump, showing only a portion of the operating lever.

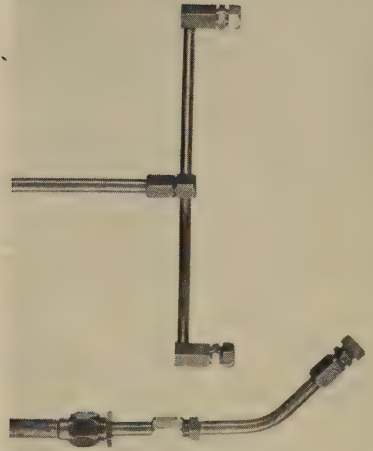


Fig. 6. Extension telescopic lance, short "broom" lance.



Fig. 7. "Trigger" tap attached to lance and fitted with $\frac{3}{4}$ " B.S.P.M. hose adaptor.



Fig. 8. Beam pump body casting showing delivery ball valves.

Acknowledgments.

The beam pump was developed and manufactured by Messrs. E. A. Gardner & Sons, Ltd., Maidstone, to whom thanks are due for their co-operation.

REFERENCE.

- (1) KEARNS, H. G. H. and MORGAN, N. G. A lightweight spray nozzle ($\frac{1}{4}$ " B.S.P.), for "small" and "large" volume spraying. *Ann. Rept. Long Ashton Res. Sta.*, 1949, pp. 111-114.

MACERATION AND DEFECATION IN CIDER-MAKING.

I. CHANGES OCCURRING IN THE PECTIN AND NITROGEN CONTENTS OF APPLE JUICES.

By F. W. BEECH and S. W. CHALLINOR.

INTRODUCTION

English and French sweet ciders differ materially in character due to the difference in national tastes, to the cider apple varieties grown and to the cider-making methods employed in the two countries. In England, the juice is usually allowed to ferment to dryness and then sweetened with cane sugar before bottling, giving a light- to medium-bodied cider in which acidity, tannin and sweetness are balanced. In France, a full-bodied, low-acid, bitter-sweet cider is often preferred (1). A naturally sweet cider is considered to have the required characteristics, and great attention is paid to the pre-treatment of the juice to assist in control of the subsequent fermentation. One method of fermentation control commonly practised in France (4) is defecation of the juice and this is often preceded by maceration of the freshly-milled apple pulp.

During the 1949-50 cider-making season, large-scale tests of the maceration and defecation treatments were carried out at the Research Station, using three of the four main types of cider apples grown in England, for the purpose of assessing :—

- (a) the effects of these treatments on the composition of the juice ;
- (b) the degree of control they afford to the subsequent fermentation ;
- (c) the quality of the ciders produced.

With the continuing shortage of cane sugar any method (such as defecation) offering a means of conserving sugar is of obvious importance.

As the processes of maceration and defecation have been described in detail in earlier reports by Charley (2) and summarised by Challinor (1) and Pollard (3), a very brief outline only will be given here. The term maceration has often been used to denote the mixing of either fresh apple pulp or once-pressed pomace with water, cider, or apple juice, but the authors prefer to regard such treatment as a diffusion process. The term maceration has been reserved to describe the process in which freshly-milled apple pulp is allowed to stand without any addition for a few hours before pressing (5).

To assist subsequent defecation small quantities of calcium carbonate and sodium chloride are added to the expressed juice which is maintained below 10° C. in either open or closed tanks. If the process of defecation proceeds satisfactorily a natural clarification occurs giving rise to a brilliantly clear juice between a brown jelly head and a deposit or "lees." In this paper the term defecation is used to describe this process which occurs after the addition of the salts. In previous Annual Reports, the defecation process has been described as "keiving," a treatment which is still used to some extent in England, but the two processes are not necessarily identical. No salts are added during "keiving" and the degree of clarification obtained depends very largely on the types of fruit used. Thus, if the apples contain large amounts of pectin and pectin esterase (pectase) then the juice submitted to "keiving" will, if left long enough, clarify naturally and a brown jelly head may form. Such a process is essentially a defecation without the addition of salts. If, however, the apples contain only small amounts of pectin and pectase, the juice remains cloudy and when fermentation begins a white or brown head of foam is formed in which a layer of brown apple pulp particles may often be found.

The clarified juice formed during the defecation process is carefully siphoned or pumped from between the brown jelly head and any "lees" which may have formed into a clean tank to ferment. The juice treated in this way has a reduced nitrogen content and microbial population and tends to ferment more slowly than the same juice untreated. Furthermore, fermentation often ceases before all the sugar has been attacked so that the cider remains naturally sweet.

A study of the principles governing maceration and defecation of French cider apple juices was started by the late Professor G. Warcollier (4). The work in France has been continued by Tavernier and Jacquin who have studied the effect of maceration and defecation on the nitrogen, sulphur and phosphorus contents of several apple juices (5). Charley (6) investigated the effects of "keiving" at two temperatures and showed that a better clarification was obtained at 2° C. than at 9° C. Studies of the action of the pectase naturally present in apple juice and of the associated pectin changes have been made by Kieser, Pollard and Stone (7) and Pollard and Kieser (8). This work demonstrated clearly that natural clarification could be effected by the enzymes present in the apple juice itself, irrespective of any enzymes elaborated by micro-organisms present in the juice. The initial concentration of pectin and pectase was shown to vary with the variety of apple. There was an indication also that the pectase concentration in the apple juice, as well as the pectin content, varied with the stage of maturity of the fruit. The activity of the pectase increased with increasing pH to a maximum approaching neutrality (pH 6.6). It was shown that successful defecation could occur at the pH values normally found in apple juice (pH 4.0 to 4.5) and Le Corvaisier (9) has shown that the defecation process can occur at as low a pH as 3.4. In general, however, the lower the pH the greater is the length of time needed for the onset of defecation, i.e. for a given concentration of pectase. At any particular pH the degree of pectase activity is also influenced by the concentration of the cations present (8). In this connexion Bejambes and Tavernier (10) have shown that an adequate concentration of calcium ions is necessary for efficient defecation, presumably in order that the pectic acid formed by the enzymic demethylation of pectin may be converted into an insoluble calcium derivative.

EXPERIMENTAL.

Cider-Making Procedure.

The varieties of cider apples used in the four series of experiments were as follows :—

Bulmer's Norman	Bittersweet	..	..	milled	10.10.49
Sweet Alford ..	Sweet	..	..	milled	24.10.49
Red Foxwhelp ..	Bitterssharp..	..	..	milled	17.11.49
Kingston Black ..	Medium Bitterssharp	..	..	milled	28.11.49

Four treatments were given in each series, namely :—

CONTROL (C). Juice from freshly-milled pulp, i.e. no special treatment.

DEFECATION (D). Juice from freshly-milled pulp, to which salts were added.

MACERATION (M). Juice from macerated pulp.

MACERATION plus DEFECATION (M + D). Juice from macerated pulp to which salts were added.

The casks (pipes) used were cleaned and steamed one hour before use, and kept in the cider-house.

Approximately two tons of sound, mature fruit were milled for each variety. One half of the pulp was pressed immediately and the juice, after bulking in a slate tank, was thoroughly mixed and tested for uniformity by carrying out determinations of specific gravity and titratable acidity on samples drawn at different depths in the tank. The juice was then distributed uniformly between a normal horizontal pipe (i.e. the Control C) and a vertical open-headed pipe for defecation (D). Defecating salts, 0.03% calcium carbonate and 0.04% sodium chloride (i.e. 4.8 ozs./100 gallons and 6.4 ozs./100 gallons respectively) were dissolved in a small quantity of the juice and added to D with thorough mixing.

Open wooden tubs 2 ft. 6 ins. deep were filled with the remaining unpressed pulp, the surface of which was then smoothed over. The pulp was allowed to macerate in the tubs for twenty-four hours and then pressed. The expressed juice was bulked and distributed as before, into a horizontal pipe (i.e. the macerated juice M) and a vertical open-headed pipe for defecation (i.e. M + D).

In order that visual observations could be made, 2½-gallon clear-glass jars were filled at this time with representative samples of juice from each pipe. Each jar stood by the side of its corresponding pipe during the remainder of the experiment.

When successful defecation occurred, the brilliant juice was siphoned from under the brown jelly head into steamed horizontal casks and allowed to ferment. Unfortunately, no means were available for controlling the temperature of the juices during the defecation process.

When the specific gravity of the fermenting macerated and defecated juice of Sweet Alford and Red Foxwhelp had fallen to S.G. 1.025, samples from the fermenting juices of the four treatments of these two varieties were removed. The remainder of the slowly fermenting juice in each cask was covered with a layer of liquid paraffin (B.P. quality) to prevent development of acetic bacteria due to the large air space caused by removal of the sample. Each sample was centrifuged using an Alfa-Laval laboratory separator, and allowed to mature for one month in filled and sealed three-gallon glass jars. Each cider after maturing was filtered through Carlson K5 sheets and divided into two equal parts; one part was left unsweetened and the other sweetened with sucrose syrup to the same specific gravity as the macerated and defecated

cider of that series. Samples of each were bottled for natural conditioning and the remaining unsweetened and sweetened ciders were artificially carbonated at 30° F. and 10 lbs. CO₂ pressure per sq. in., sterile-filtered through Carlson EK. sheets, bulked and bottled. All the ciders were bottled in champagne pints, previously sterilised with 2% sulphur dioxide solution, and stored in a cool cellar for approximately six months before tasting.

Visual and Other Observations.

Maceration.

The system of maceration employed (i.e. relatively deep layers) was essentially anaerobic and according to Warcollier (4), a greater extraction of pectin and pectase occurs under such conditions without the simultaneous removal, by oxidation, of a large proportion of the tannin from the expressed juice. Aerobic maceration for the same length of time would have caused extensive oxidation and encouraged acetification. The pulp in the tubs after the period of maceration was free from obvious acetification and was apparently only slightly oxidised, i.e. browning occurred at the surface and below to a depth of one inch. The remainder appeared to be slightly lighter in colour than the original unmacerated pulp.

Table I shows analyses carried out on the fresh juices from unmacerated and macerated pulps.

TABLE I.
EFFECT OF MACERATION ON THE "TANNIN" AND
VOLATILE ACIDITIES OF THE EXPRESSED JUICE.
(Results expressed as g. per 100 ml. of juice).

		Bulmer's Norman	Sweet Alford	Red Foxwhelp	Kingston Black
" Tannin "	Before Maceration (C)	0.35	0.13	0.20	0.14
	After Maceration (M)	0.32	0.12	0.18	0.10
Volatile Acidity (as acetic acid)	Before Maceration (C)	—	—	0.035	0.011
	After Maceration (M)	—	—	0.032	0.013
Duration of Maceration		14 hours	22 hours	16 hours	27 hours

With the exception of Kingston Black in which the pulp was held in the tubs for the greater length of time, maceration had little significant effect on the tannin of the juice subsequently expressed. Volatile acidity did not alter appreciably, i.e. for either of the two varieties examined.

Defecation.

While one of the objects of defecation is to secure a clarified juice, in practice the most obvious sign of successful treatment is the formation of a

compact, brown, pectin-jelly head and it is necessary to sample the juice under the head before the degree of clarification can be assessed. Plate XIII, Fig. 1 shows a typical brown jelly head formed during successful defecation and Plate XIII, Fig. 2 shows a white head as formed by the Controls and other juices which did not defecate.

A summary of the defecation treatments that gave rise to brown jelly heads and to clarification is given in Table II together with the time taken for the heads to form. All other treatments gave white heads and the juices did not clarify.

TABLE II.
TIME TAKEN FOR DEFECATION TO OCCUR.

Variety	Treatment	Time taken for the formation of a brown head
Bulmer's Norman ..	Defecation only (D)	2 days
	Maceration plus Defecation (M + D)	1 „
Sweet Alford ..	Defecation only (D)	3+4 „
	Maceration plus Defecation (M + D)	2 „
Red Foxwhelp ..	Defecation only (D)	7 „
	Maceration plus Defecation (M + D)	3 „
Kingston Black ..	Maceration plus Defecation (SO ₂ treated) juice (M + D + SO ₂)	18 „

Notes : 1. Three days after pressing, the Sweet Alford juice to which defecating salts had been added (D), formed a white foam head above a cloudy juice. This juice was siphoned into a clean, freshly steamed, horizontal cask and four days later the juice extruded a large quantity of brown, pectin-jelly clots through the bung-hole of the cask, leaving a brilliant juice.

2. With Kingston Black, defecation was very slow and only macerated and defecated juice to which 60 p.p.m. SO₂ had been added initially to restrict fermentation, eventually developed a brown head 18 days after pressing. However, the head that formed was very thin and loose and the juice underneath was not brilliant.

Analytical Methods.

The following determinations were made on samples of the fermenting juices and ciders at suitable intervals and references are given for the methods employed :—Specific Gravity (14); Titratable acidity (11); Volatile acidity (12 and 13); pH (glass electrode); "Tannin" (12); Rate of Fermentation (14); Sulphur Dioxide (13); Total Reducing Sugars (14).

Nitrogen : Determined by micro-Kjeldahl, expressed as mg.N/100 ml. (14). Soluble nitrogen was determined after centrifuging at 2,500 p.p.m. for 30 mins.

Pectin : Determined by Carré and Haynes method on centrifuged juice before and after precipitation with acetone (15). Expressed as calcium pectate g./100 ml.

RESULTS.

For each variety, the total quantity of fruit needed to provide the juice for all four treatments was milled on the same day, and half of the milled pulp was pressed immediately for the Control and Defecation treatments. The juice from the macerated pulp was not expressed until the following day, i.e. for the Maceration and Maceration plus Defecation treatments. Since the remaining treatments were carried out on the juice, the *date of pressing* was regarded as the start of each treatment and the time-references in text, tables and figures have been reckoned from that date.

Pectin Changes due to Maceration and Defecation.

Analyses of both crude and purified pectin were carried out on Sweet Alford and Red Foxwhelp juices. The analyses showed the same trends for all treatments although the crude pectin was always slightly greater in amount. Maceration of the pulp had no effect on this difference in either variety. All estimates of pectin quoted in the remainder of this section relate only to purified pectin.

In Table III are given the pectin contents of the juices freshly expressed from the unmacerated and macerated pulps of Sweet Alford, Red Foxwhelp and Kingston Black.

TABLE III.
EFFECT OF MACERATION ON THE PECTIN CONTENT OF FRESH JUICES.
(Results expressed as g.calcium pectate per 100 ml. juice).

Variety	Pectin Content		Change in Pectin Content
	Before Maceration	After Maceration	
Sweet Alford ..	0.047	0.072	+0.025
Red Foxwhelp ..	0.090	0.139	+0.049
Kingston Black ..	0.077	0.089	+0.012

The initial pectin content of the fresh juices differed for the three varieties. Maceration of the pulp caused a significant increase in the pectin of the expressed juice in each variety. Sweet Alford and Red Foxwhelp showed the greatest increase and in these varieties, the subsequent defecation was successful.

The pectin changes that followed the different juice treatments are shown for Sweet Alford in Table IV.

TABLE IV.
EFFECT OF DEFECACTION ON THE PECTIN CONTENT OF FRESH JUICE.
SWEET ALFORD.

Treatment	pH of Juice After Treatment	Pectin Content (g. calcium pectate per 100 ml. of juice)			Rate of Pectin Removal per day (as mg. Calcium pectate per 100 ml. juice)
		At Time of Pressing	2 Days After Pressing	7 Days After Pressing	
No Treatment (C)	4.1	0.047	0.039	0.019	4
Defecation only (D)	4.4	0.047	0.025+	None*	11
Maceration only (M)	4.1	0.072	0.057	0.030	7
Maceration plus Defecation (M + D)	4.4	0.072	None*	None	36

+ Estimation carried out on juice siphoned off from under the white head.

* Estimation carried out on clarified juices after successful defecation.

It is evident that the process of defecation effectively reduced the pectin content of the treated juices; thus, in the defecated juices D and M + D, pectin was absent, e.g. after only two days for M + D. The rates of pectin removal for the first few days after the four treatments have been calculated and are shown in the right hand column. In the juices submitted to defecation, D and M + D where ample calcium ions were added initially, the increased rate of pectin removal in the juice from macerated pulp (M + D), as compared with the juice from unmacerated pulp (D), indicated a greater extraction of pectase from the pulp during maceration. This was also suggested, but to a lesser extent, by the corresponding figures for the non-defecated juices (C and M), the rate of pectin removal being greater after maceration. The slower rate of pectin removal in the non-defecated juices as compared with defecated juices, may be ascribed either to a deficiency in calcium ions or to the lower pH. The successful defecation of Red Foxwhelp juice at the low pH of 3.3 would, however, suggest that the pH is not the decisive factor for this juice.

Despite an unfavourable pH (average 3.3), the rate of pectin removal in Red Foxwhelp juice was slightly greater for each of the four treatments than in Sweet Alford juice. A possible explanation of these results is that Red Foxwhelp contained a larger concentration of pectase than Sweet Alford. Other tests showed that the fruit sample of Red Foxwhelp was in fact high in pectase.

Nitrogen Changes due to Maceration and Defecation.

Samples for analysis were withdrawn from the centre of each pipe without disturbing the lees; thus, the analyses relate to the fermenting juice and the nitrogen content of the lees is not included. The following interpretation of the nitrogen data and its conversion into data representative of yeast,

is that used by Burroughs and Challinor (16). This conversion is true provided the micro-organisms present consist almost entirely of yeasts and do not include more than a small proportion of bacteria. From the few microscopical observations carried out on each of the fermentations of the Sweet Alford juice, it appeared that the material in suspension did in fact consist almost entirely of yeasts.

Thus, for the Controls in each of these experiments, i.e. the untreated juices which were allowed to ferment naturally, the total nitrogen in any sample is made up of the soluble nitrogen plus nitrogen in suspension. Nitrogen in suspension, which may be regarded as representative of yeast in suspension in any particular sample, is obtained by subtracting the soluble nitrogen from the total nitrogen of that sample. Nitrogen in the lees, regarded as representative of yeast in the lees at any time, is not determined directly but is obtained by subtracting the total nitrogen at the time of sampling from the initial total nitrogen of the juice. An indication of the amount of yeast which has grown up to any particular time is given by subtracting the soluble nitrogen at that time from the initial soluble nitrogen of the fresh juice.

Nitrogen Changes in the Untreated Juices (i.e. Controls).

These changes in the distribution of the nitrogen, regarded as representative of the changes in yeast distribution, are shown diagrammatically in Fig. 3, using data obtained from the fermentation of the Control Sweet Alford juice, since this fermentation was examined more fully in this respect than the other fermentations. All nitrogen data have been expressed as mg. N/100 ml. of juice or cider and have been abbreviated to mg. in the text.

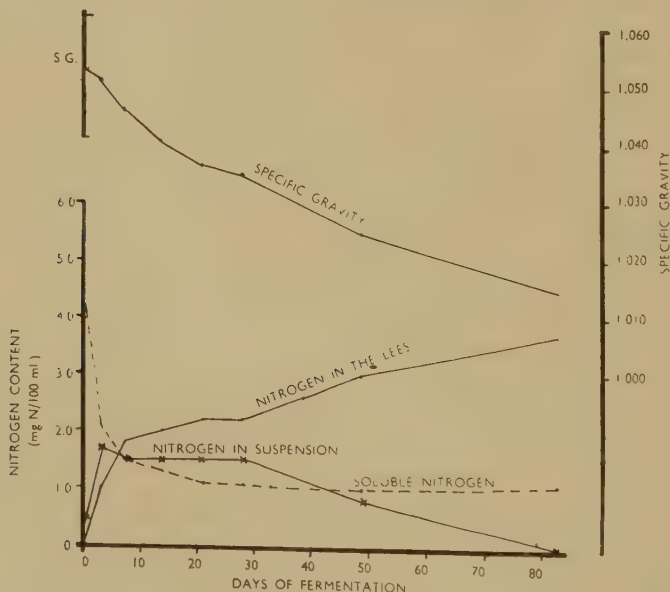


Fig. 3. Fermentation of the Control (Untreated) Sweet Alford Juice showing changes in Specific Gravity and Distribution of Nitrogen in the Fermenting Juice.

During the first eighty days of this fermentation, the specific gravity decreased from an initial S.G. 1.053 to S.G. 1.016. The rate of fermentation during the first three days was slow, a fall of two degrees in S.G., but the soluble nitrogen of the juice decreased very considerably, from 4.4 mg. to 2.1

mg., i.e. a loss of 52%, indicating an extremely rapid utilisation of soluble nitrogen for yeast growth. Soluble nitrogen decreased more slowly to 1.1 mg. from the 3rd to the 21st day, i.e. down to 25% of the initial soluble nitrogen content. Thus, during the first part of the fermentation, occupying twenty-one days, although there was a loss of only 16 degrees in specific gravity, representing one-third of the total decrease in S.G., the greatest utilisation of soluble nitrogen occurred and the remainder of the fermentation proceeded with practically no further decrease in this fraction.

Nitrogen in suspension increased rapidly to a maximum of 1.7 mg. on the 3rd day and then after a very slight fall, remained steady at 1.5 mg. for a further twenty-one days. From the 28th day onwards, there was a steady decrease in the amount of this fraction and on the 83rd day it was negligible. Nitrogen continued to accumulate in the lees throughout the eighty-three days, but the rate of accumulation for the first seven days was very much faster, approximately eight times, than it was from the 7th to the 83rd day. Thus, in a Control fermentation, loss of soluble nitrogen is representative of growth of yeast, which either remained in suspension or accumulated in the lees; this nitrogen remained in the fermentation vessel.

Nitrogen Changes Due to Maceration.

The Sweet Alford juice freshly expressed from macerated pulp had a slightly lower soluble nitrogen content than juice from unmacerated pulp; 4.0 mg. and 4.4 mg. respectively, i.e. a difference of 9%. In the juices of the other three varieties however, the soluble nitrogen increased slightly after maceration, as illustrated by the nitrogen-data for Red Foxwhelp shown in Table V.

TABLE V.
EFFECT OF MACERATION ON THE NITROGEN CONTENTS OF FRESH JUICE.
RED FOXWHELP.

(Results expressed as mg.N/100 ml. juice).

Nitrogen	Before Maceration	After Maceration	Change in Nitrogen Content
Total	2.5	2.9	+0.4
Soluble	2.3	2.5	+0.2
In Suspension ..	0.2	0.4	+0.2

Thus in this variety there was a 9% increase in soluble nitrogen following maceration of the pulp; in Bulmer's Norman there was also a 9% increase, i.e. from 16.9 mg. to 18.4 mg.; in Kingston Black there was a 12% increase from 1.7 mg. to 1.9 mg. Tavernier and Jacquin (5) reported no change after maceration; they, however, did not specifically estimate soluble nitrogen, but carried out all their nitrogen analyses on juices which had been strained through fine silk.

Nitrogen Changes which take place during the Defecation Process.

These changes are illustrated in Table VI. Thus it is seen for Sweet Alford

TABLE VI.
NITROGEN CHANGES DURING DEFECTION.
SWEET ALFORD AND RED FOXWHELP.
(Results expressed as mg.N/100 ml. Juice).

Treatment	Nitrogen	SWEET ALFORD				RED FOXWHELP			
		Freshly Expressed Juice	Clarified Juice, i.e. after Defecation	Change in Nitrogen Content	Percentage Change	Freshly Expressed Juice	Clarified Juice, i.e. after Defecation	Change in Nitrogen Content	Percentage Change
Defecation Only (D)	Total ..	4.8	2.3	-2.5	-52%	2.5	1.6	-0.9	-36%
	Soluble ..	4.4	1.7	-2.7	-61%	2.3	1.3	-1.0	-43%
	In Suspension	0.4	0.6	+0.2	+50%	0.2	0.3	+0.1	+50%
Maceration and Defecation (M + D)	Total ..	4.8	2.0	-2.8	-58%	2.9	2.0	-0.9	-31%
	Soluble ..	4.0	1.7	-2.3	-58%	2.5	1.6	-0.9	-36%
	In Suspension	0.8	0.3	-0.5	-63%	0.4	0.4	0.0	nil

and Red Foxwhelp, where defecation was successful, that there was a considerable *decrease* in the soluble nitrogen content of the juices as a result of defecation. The decreases in soluble nitrogen which occurred in the untreated juices, i.e. Controls, to which reference has already been made were also considerable but, as explained in the Discussion, have an entirely different significance.

Changes observed in the nitrogen in suspension were irregular, small in amount and call for no special comment.

Nitrogen Changes Subsequent to Defecation.

For Sweet Alford and Red Foxwhelp, changes in soluble nitrogen during the fermentation of defecated juices from both unmacerated and macerated pulp, i.e. D and M + D, were only slight in extent. In contrast, changes in soluble nitrogen during the fermentation of juices that had not been submitted to defecation, i.e. C and M, were very large. The nitrogen changes for the fermentations of the juices from macerated Sweet Alford pulp, i.e. both M and M + D, are illustrated in Fig. 4.

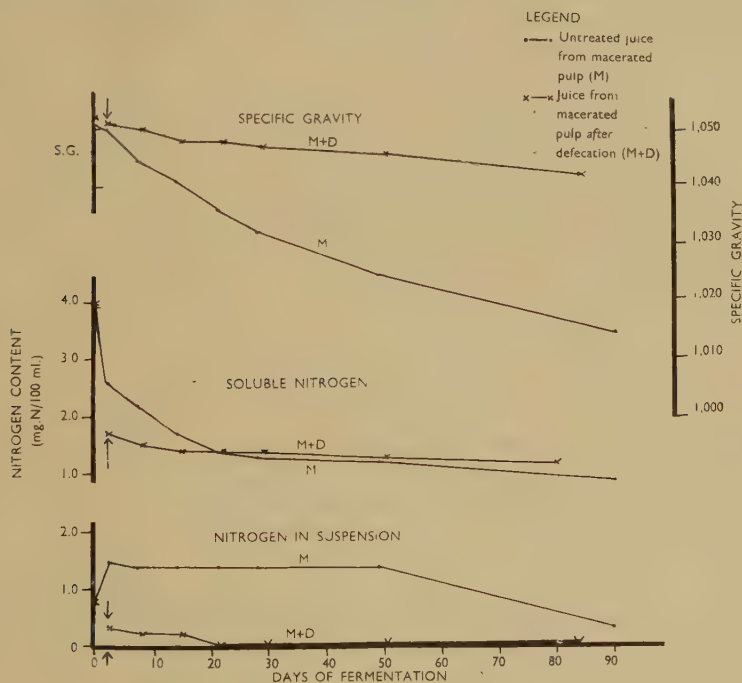


Fig. 4. Fermentations of the Juices from Macerated Pulp of Sweet Alford. Specific Gravity Curves and Changes in Soluble Nitrogen and Nitrogen in Suspension.

The arrow $\uparrow$ indicates the day on which the defecated juice (M + D) was siphoned off from the brown head and lees.

For comparison the macerated juice will be considered first. In this juice, as illustrated in Fig. 4, there was a large decrease in soluble nitrogen, from 4.0 mg. to 1.4 mg. in the first twenty-one days, as a result of the rapid growth of yeast which occurred; during this time there was a large decrease in specific gravity (15 degrees). Subsequent decreases in soluble nitrogen were small, only 0.4 mg., so that after twenty-one days there was little further

growth of yeast. However, the specific gravity continued to decrease a further 21 degrees over the remaining fifty-nine days.

In contrast to the large change in soluble nitrogen which occurred during the fermentation of the macerated juice, the corresponding changes in the juices which defecated were (as stated above) very small. Thus, in eighty days there was a loss of only 0.5 mg. soluble nitrogen, indicating that very slight growth of yeast took place during this period. Further evidence that growth of yeast was slight is given by (i) the small amounts both of nitrogen in suspension, which is regarded as representative of yeast in suspension, and of nitrogen in the lees, which is representative of yeast in the lees, and by (ii) the slow rate and restricted extent of the fermentation, e.g. a decrease of only 9 degrees of specific gravity during the eighty days as compared with a decrease of 34 degrees in the macerated, non-defecated juice (M), for this period.

It may be observed from Figs. 3 and 4 that if the untreated juices C and M, which did not defecate, had been siphoned into fresh casks at the same time as the treated juice M + D, large amounts of yeasts would still have been left in suspension. Thus the subsequent fermentation of these untreated juices would not have been greatly restricted.

The Effects of these Treatments on Fermentation and on the Stability of the Resulting Ciders.

The main object of the process of defecation is the production of naturally sweet ciders. Thus, the defecation treatment reduces both the rate and extent of the fermentation so that fermentation either ceases while the cider is still naturally sweet or is so slow that artificial control, e.g. by centrifuging, can be exercised effectively.

The effect of the treatments on the rate and extent of fermentation of three juices is illustrated in Table VII. The fourth juice from Kingston Black did not respond to the defecation process.

TABLE VII.
EFFECT OF MACERATION AND DEFEICATION ON THE RATE OF FERMENTATION.
BULMER'S NORMAN, SWEET ALFORD AND RED FOXWHELP.

Treatment	BULMER'S NORMAN		SWEET ALFORD		RED FOXWHELP	
	Rate of Fermentation at 25° C. in the laboratory	Time to Ferment to S.G. 1,025 in the Ciderhouse	Rate of Fermentation at 25° C. in the laboratory	Time to Ferment to S.G. 1,025 in the Ciderhouse	Rate of Fermentation at 25° C. in the laboratory	Time to Ferment to S.G. 1,025 in the Ciderhouse
No Treatment* .. (C)	7.8	7 days	1.1	48 days	0.7	54 days
Defecation only+ .. (D)	4.4	17 days	—	90 days	†	†
Maceration only* ... (M)	7.8	6 days	1.3	51 days	0.8	101 days
Maceration + Defecation+ (M + D)	2.3	30 days	0.6	180 days	0.3	171 days

* i.e. the fresh juices.

+ i.e. juices which have responded to the defecation process.

† Still at S.G. 1,040 one year after pressing the pulp. S.G. of juice before defecation was 1,053.

It will be seen that the preliminary defecation treatment has greatly reduced the rate of fermentation. In each of the three varieties the rates of fermentation of the defecated juices, i.e. D and M + D, were slower than those of the corresponding non-defecated juices C and M. The effects of maceration on the rates of fermentation were not consistent throughout the three series so that no definite conclusion can be drawn at present.

The extent of fermentation in the defecated juices varied considerably. Only the defecated juice of Red Foxwhelp ceased fermentation while still naturally sweet and this juice commenced fermentation with the lowest soluble nitrogen content (1.2 mg.) of any of the varieties. Although this juice after maceration plus defecation eventually fermented to dryness, this did not occur until after ten months, whereas the untreated juice, i.e. the Control, was dry after six months. In contrast, both the defecated juices of Bulmer's Norman fermented rapidly to dryness. However, in the juice of this variety the initial soluble nitrogen (and probably other yeast nutrients also) was large and presumably sufficient remained after defecation to support growth of yeast in quantity, so that fermentation was rapid. With Sweet Alford the juice after maceration plus defecation developed sickness at specific gravity 1.023 and fermented to dryness; this juice after defecation only, which had reached S.G. 1.008 at this time, did not develop the disorder and fermented to dryness normally. It was observed that the titratable acidity of the cider which developed the disorder (i.e. M + D) remained much lower than the titratable acidities of the other three ciders of this variety. The development of the bacteria would probably have been inhibited if this cider from macerated and defecated juice had been blended earlier with high-acid cider, as in the normal commercial treatment of low-acid juices. This, in fact was not done in these experiments since the ciders were kept deliberately as single varieties in order that their individual fermentations could be studied.

The Effects of these Treatments on the Quality of the Ciders.

The ciders will be referred to in this section according to the treatments originally given to the juices from which they were made, e.g. the Control cider refers to a cider resulting from the fermentation of untreated juice. Similarly the Macerated and Defecated cider refers to the cider made from the defecated juice obtained after maceration of the pulp.

Only the ciders made from Sweet Alford and Red Foxwhelp, i.e. those in which successful defecation occurred, were sampled for bottling (see Experimental, Cider-Making Procedure).

The quality of each cider was assessed by the procedure reported elsewhere (12). Only a summary of the most important features found in each cider will be given.

Naturally Conditioned Ciders.

It was difficult to assess, the quality of the naturally conditioned ciders since, with the exception of the Macerated and Defecated cider from Sweet Alford, which was "sick," none of these ciders developed any perceptible degree of carbonation. This lack of conditioning, even in the sweetened ciders was probably due to the fact that they were bottled in the late summer; bottling of the ciders of each variety was delayed until the Macerated and Defecated cider of that variety had fermented to S.G. 1.025.

Artificially Carbonated (Sterile-filtered) Ciders.

The uniform carbonation of the sterile-filtered ciders simplified the assessment of their quality considerably.

The Control ciders of Red Foxwhelp, both unsweetened (S.G. 1,003-5), and sweetened (S.G. 1,022) had a peculiar resinous aroma which persisted to a smaller extent in the flavour, spoiling an otherwise pleasant and fruity cider. The resinous character, however, was not found in the Defecated cider (S.G. 1,041) of this variety which, although high in acid and tannin, also had a high natural sugar content so that the cider was balanced in flavour. This clean, fruity, pleasant cider was given the highest figure of merit of the ciders made from Red Foxwhelp. All the ciders made from the macerated pulp of Red Foxwhelp possessed an intense, almost quinine-like bitterness which was unpleasant. The unsweetened Macerated cider (S.G. 1,006-5) possessed a fruity aroma, while the aroma of the sweetened cider (S.G. 1,021-5) was slightly resinous. The Macerated and Defecated cider (S.G. 1,021-5) was unpleasant since it had both the resinous aroma and intense bitter flavour.

All but one of the ciders made from Sweet Alford were clean in aroma and flavour. The Control cider unsweetened (S.G. 1,001) was a pleasant, dry cider, whereas the same cider sweetened (S.G. 1,021) was in addition, fruity and typical of the variety. The effect of sweetening in improving the flavour was even more marked in the Defecated ciders, where the unsweetened cider (S.G. 1,001) had little body or character but when sweetened (S.G. 1,021) possessed these qualities in good measure comparable with the sweetened Control cider. The unsweetened Macerated cider (S.G. 1,003-5) was given a slightly greater figure of merit than any other cider made from Sweet Alford, having more acidity, a pleasant tannin bitterness and fruity character; when sweetened (S.G. 1,021) it was well balanced in flavour. The quality of the Macerated and Defecated cider (S.G. 1,021) could not be assessed since the characteristic aroma and flavour of "sickness" was present in slight amount. This cider must have been slightly "sick" when it was sterile-filtered since there were no signs of bacterial fermentation having taken place in the bottle.

Thus the Control ciders, both sweetened and unsweetened, from Sweet Alford were of high quality, whereas the Control ciders from Red Foxwhelp were poor. Maceration of the Sweet Alford pulp apparently improved the quality of the cider but with Red Foxwhelp the same treatment gave a cider inferior to the Control. The quality of the ciders made from macerated and defecated juices, i.e. M + D, could not be assessed, since this cider from Sweet Alford was slightly "sick" and the corresponding cider from Red Foxwhelp had the resinous quality of the fresh juice combined with the intense bitterness due to maceration of the pulp. Defecation of the juice from freshly-milled Sweet Alford pulp gave a cider which, after sweetening, was of high quality; the same cider from Red Foxwhelp was naturally sweet and better than the corresponding Control cider.

DISCUSSION.

In this present work, four typical varieties of cider apples have been studied in relation to their behaviour during the processes of Maceration and Defecation. The results will be summarised first, and then followed by a discussion in more detail.

- (a) The four varieties varied in the extent to which they responded to the two treatments.

- (b) The process of maceration increased the pectin content of the juice, the increase varying according to the variety of apple. The varieties which showed the largest increase of juice pectin due to maceration also clarified most rapidly during the subsequent defecation.
- (c) Successful defecation was accompanied by complete precipitation of pectin from solution.
- (d) During the course of defecation there was a considerable reduction of soluble nitrogen in the juice. The rates and extents of the subsequent fermentations of the defecated juices showed some relation to the changes in soluble nitrogen that took place during defecation.

(a) Defecation did not occur in any of the four varieties without the addition of salts. The defecated juices, i.e. D and M + D treatments, of the three varieties, Bulmer's Norman, Sweet Alford and Red Foxwhelp showed rapid clarification; the fourth variety did not clarify satisfactorily. Warcollier (4) and Tavernier (5) have shown in this connection that the maturity of the fruit as well as the variety is also important. Thus, it is not possible to say at present whether these varieties will behave in the same manner each year.

(b) For the three varieties in which pectin changes were followed, maceration caused an increase in the pectin content of the expressed juice; the increase varied according to the variety. It was found that where the pectin increase was greatest and of the order of 50%, as in Sweet Alford and Red Foxwhelp, the subsequent clarification of the macerated and defecated juices was satisfactory. This would suggest that there was an increase in the pectase content of the juice accompanying the large increase in pectin and this is further borne out by the more rapid removal of pectin which occurred in the juice prepared from macerated pulp as compared with juice from unmacerated pulp (Table II). The increase in pectin may itself be due to the action of the enzyme pectase during maceration.

(c) Successful defecation was characterised by the rapid formation of a brown, pectin-jelly head and the complete and rapid removal of soluble pectin from solution. For example, pectin was no longer detectable in the macerated and defecated juice from Sweet Alford after two days.

It is interesting to compare this process, carried out under controlled conditions, with the phenomenon of "dropping bright" which occurs occasionally in the normal course of cider-making. In this phenomenon, which has been discussed in an early paper by Charley (17), a pectin clot is formed, giving rise to a brilliantly clear juice, which, if racked, ferments very slowly and may cease fermenting while still naturally sweet. It may be supposed that such apple juices contain large amounts of pectase and sufficient calcium ions to precipitate the pectin as a pectate gel. In a normal fermentation a gradual clarification usually occurs and has been found in other experiments to be accompanied by a slow removal of pectin. The influence of pH and of the concentrations of salts in such clarifications will require further investigation, for it is evident from the present experiments that addition of defecating salts does accelerate the course of juice clarification (Table IV).

(d) The soluble nitrogen content of the juice is one of the main factors determining the course of the subsequent fermentation. As emphasised earlier, one of the main effects of defecation is the removal of nitrogen from the juice. In fact, the percentage removal of soluble nitrogen was very great and of the

order of 50% in the defecated juices of Sweet Alford and Red Foxwhelp. Consequently the amount of soluble nitrogen remaining for yeast growth in the defecated juice was considerably reduced. For example, when the percentage removal of soluble nitrogen during defecation was greatest (Sweet Alford and Red Foxwhelp) there was a very considerable restriction of fermentation in the defecated juices; in Bulmer's Norman where the percentage removal was less, although the fermentation was restricted compared with the Control of that variety, the juice fermented to dryness in forty days. The importance of the amount of nitrogen still remaining in the defecated juices has been discussed by Tavernier (18) who states that if the ratio

$$\frac{\text{Total Nitrogen mg./litre}}{\text{Total Reducing Sugars g./litre}}$$
 for a defecated juice is not greater than 0.4, then the fermentation will cease while natural sugar still remains. For Bulmer's Norman the clarified juices after defecation fermented rapidly to dryness and this ratio for the M + D treatment was 1.2. For Sweet Alford and Red Foxwhelp, there was considerable restriction of fermentation, and the ratio for the same treatment, i.e. M + D, was 0.18. This ratio for the defecated juice of Red Foxwhelp which eventually ceased fermentation while naturally sweet was 0.15. It must be emphasised however, that the ratio for the Control juices of Sweet Alford and Red Foxwhelp were themselves only 0.4 and 0.23 respectively. While it is true that a juice with a ratio greater than 0.4 may not remain naturally sweet, these experiments show that the converse is not necessarily true since the juices with a ratio less than 0.4 (with the exception of the defecated Red Foxwhelp juice) whether defecated or not, did not remain naturally sweet. Thus the differences in the extent of fermentation observed even in the juices in which this ratio was low, cannot be explained entirely by the nitrogen contents of the defecated juices. In fact, the variations in behaviour showed by the defecated juices of Sweet Alford and Red Foxwhelp were not entirely consistent with the amounts of nitrogen remaining in the juice. Further investigation may show that only certain nitrogenous constituents may be concerned in this connexion and or that deficiencies of other yeast nutrients may also be concerned.

The mechanism whereby soluble nitrogen was removed from the juice was not investigated specifically in these experiments and must be the subject of further research. Tavernier and Jacquin (5) state that defecation converts the greater part of the soluble proteins and polypeptides of the juice into insoluble compounds which are removed in the brown, pectin-jelly head. They state that these nitrogenous constituents are essential for yeast growth, and that in consequence, such a juice could only ferment very slowly and often ceased to ferment while the juice was still naturally sweet. However, Thorne (19) and other workers who have investigated the assimilation of different nitrogenous constituents by yeast, have shown that the yeasts they were examining, utilised single amino acids more readily than simple peptides and much more readily than polypeptides. A possible mechanism suggested by the work reported in this present paper is as follows: When the juice is not submitted to defecation, a rapid growth of yeast takes place: such growth of yeast did in fact occur during the "lag" period of the normal fermentation of these untreated juices, i.e. the Controls, depleting the juices not only of nitrogen but also of other yeast nutrients (see Burroughs and Challinor (20)). In the defecated juices any yeast crop would be removed in the brown jelly head or lees. It has been generally assumed that the brown jelly head plays a purely mechanical part in removing the suspended material,

whether it consists of "precipitated polypeptides" or of yeast cells, but chemical adsorption between the precipitated pectin complex and the amino acids of the juice may also occur.

The effects of these different treatments on the quality of the ciders made from Sweet Alford and Red Foxwhelp have already been discussed in an earlier section of this paper and only a brief summary of the more important findings will be made here. For these two varieties, the ciders made after defecation of the juices were of high quality; in particular the naturally-sweet cider from this treatment of Red Foxwhelp juice was of much higher quality than the corresponding untreated, Control cider. From the limited experience obtained in these experiments involving maceration in cider-making it is not possible to generalise about its effect on the final cider, for while anaerobic maceration of the low-acid, Sweet Alford pulp was beneficial, maceration of the highly acid, bittersharp, Red Foxwhelp pulp was detrimental since the tannin bitterness was unpleasantly emphasised.

The production of a naturally sweet cider by preliminary defecation of the juice demands very close control both of the juice and of the fermentation at all stages and a clear understanding of the processes involved (see also Warcollier (4)). To facilitate this control, the cider-making procedure and factory lay-out employed must be specially designed, if the process is to be operated economically on a large scale. Thus, the milled pulp should preferably be allowed to fall into small tanks above the presses for maceration. After maceration the pulp should then fall by gravity to the cheese-building platform. Similarly, the tanks for the defecation process should be totally enclosed, and fitted with observation windows and attenuator coils, so that the temperature of the juice undergoing defecation can be controlled to ensure that pectin clotting is complete before fermentation commences.

Irrespective of the quality of the resulting ciders or the degree of control involved in carrying out the processes, this study of maceration and defecation and the subsequent fermentations has given further insight into fundamental aspects of cider-making in general. For a more complete understanding of the mechanism of the defecation process, it will be necessary to supplement these results by qualitative examination of the types of nitrogenous constituents present in the juice initially and after the process of defecation has occurred.

SUMMARY.

1. An investigation has been made of the effects of maceration and defecation on the juices of four varieties of cider apples.
2. The four varieties varied in the extent to which they responded to the two treatments. Three of the four juices clarified and responded well to the treatment.
3. Maceration increased the pectin and pectase contents of the expressed juices and thus facilitated subsequent defecation.
4. Successful defecation was accompanied by complete precipitation of pectin from solution.
5. The soluble nitrogen contents of the juices which responded to defecation were thereby greatly reduced.
6. The rates and extents of the subsequent fermentations of the defecated juices showed some relation to the changes in soluble nitrogen that occurred as the result of defecation, although the results suggest that factors other than soluble nitrogen may also be concerned.

7. An assessment has been made of the quality of the ciders produced after these treatments. Ciders made from defecated juices were of high quality. The effects of maceration on the quality of the ciders varied with the variety.
8. An indication has been given of some of the practical cider-making aspects of these two processes.

Acknowledgments.

We wish to express our thanks to Messrs. J. H. Llewellyn and R. J. Hathway for assistance in the Cider-House, and to Miss Yvonne P. May and Mrs. Barbara A. Baker for assistance with analytical work.

REFERENCES.

- (1) CHALLINOR, S. W. *Ann. Rept. Long Ashton Res. Stn.*, 1948, 216.
- (2) CHARLEY, V. L. S. *Ibid.*, 1937, 160.
- (3) POLLARD, A. *Ibid.*, 1948, 222.
- (4) "The Principles and Practice of Cider-Making," a translation by DR. V. L. S. CHARLEY of "La Cidreterie" by PROF. G. WARCOLLIER. Chapters VI and IX.
- (5) TAVERNIER, J. and JACQUIN, P. *Comptes rend., Acad. Sci.*, 1946, **222**, 416.
- (6) CHARLEY, V. L. S. *Ann. Rept. Long Ashton Res. Stn.*, 1935, 138.
- (7) KIESER, M. E., POLLARD, A. and STONE, A. M. *Ibid.*, 1948, 228.
- (8) POLLARD, A. and KIESER, M. E. *J. Sci. Food Agric.*, 1951, **2**, 30.
- (9) LE CORVAISIER, H. *Chim. et Ind.*, 1946, **56**, 382.
- (10) BEJAMBES, M. and TAVERNIER, J. *Bull. Assoc. Chim. Sucr.*, 1945, **62**, 135.
- (11) BURROUGHS, L. F. *Ann. Rept. Long Ashton Res. Stn.*, 1946, 127.
- (12) CHALLINOR, S. W. and BURROUGHS, L. F. *Ibid.*, 1947, 172.
- (13) *Methods of Analysis of A.O.A.C.* (6th Edition), 1945, Chapter XV.
- (14) BURROUGHS, L. F. and CHALLINOR, S. W. *Ann. Rept. Long Ashton Res. Stn.*, 1949, 115.
- (15) HINTON, C. L. *Fruit Pectins*, D.S.I.R. Special Report, No. 48, 1939, Part I.
- (16) CHALLINOR, S. W. and BURROUGHS, L. F. *Ann. Rept. Long Ashton Res. Stn.*, 1948, 182.
- (17) CHARLEY, V. L. S. *Ibid.*, 1934, 217.
- (18) JACQUIN, P. and TAVERNIER, J. Extract du procès-verbal de la Seance, Académie D'Agriculture de France, 24th January, 1951.
- (19) THORNE, R. S. W. *Wallerstein Labts. Commun.*, 1950, **13**, 319.
- (20) BURROUGHS, L. F. and CHALLINOR, S. W. *Ann. Rept. Long Ashton Res. Stn.*, 1950, 161.



Fig. 1. Typical brown, pectin jelly head formed, bringing successful defecation.



Fig. 2. Typical "white head" formed during the early stages of fermentation of juices that did not defecate.

THE MECHANISM OF THE CONTROL OF FERMENTATION OF CIDERS BY CENTRIFUGING.

By L. F. BURROUGHS and S. W. CHALLINOR.

INTRODUCTION

In a previous paper (2) the fermentation of a Bramley juice was checked at different specific gravities by centrifuging and the stability of the resulting ciders was examined. Varying degrees of stability were obtained and an attempt was made to explain the mechanism of this method of controlling fermentation by a study of the changes in the nitrogen content of the fermenting juices which occurred prior to centrifuging (see also Ref. 1). It was suggested that effective control of the fermentation may be expected if the juice is centrifuged after utilisation of the readily available soluble nitrogen† is complete, but that some factor other than nitrogen might also be involved.

Phosphorus is known to be an essential yeast nutrient and to play an important part in fermentation. A detailed study has been made of the changes in both the nitrogen and phosphorus contents of a Bramley juice during its undisturbed fermentation; this work was described in the previous Annual Report (3). It was shown that the rate of utilisation of the soluble nitrogen of this juice, which may be regarded as representative of the rate of growth of yeast, was rapid and was uniform during the period when yeast growth was most active. Rate of utilisation of soluble nitrogen subsequently became slower as the soluble nitrogen content of the fermenting juice decreased to a minimum value; this was followed by a gradual increase in soluble nitrogen due to autolysis of the yeast. The course of the utilisation of soluble phosphorus was very similar to that observed for soluble nitrogen, i.e. soluble phosphorus decreased rapidly, then more slowly to a minimum value after which it increased, the increase being relatively greater than the corresponding increase in soluble nitrogen. It is apparent that utilisation either of soluble nitrogen or of soluble phosphorus can each be regarded as representative of growth of yeast. Furthermore, the time at which soluble nitrogen and soluble phosphorus reached their observed minimum values can be regarded (approximately*) as marking the cessation of yeast growth.

The work described in this present paper concerning the mechanism of the control of fermentation by the centrifuge, was done on replicate casks of the same Bramley juice, the undisturbed fermentation of which was studied in the previous paper (3). In the present experiment the fermentation of this juice was checked by centrifuging at different specific gravities and the resulting ciders were stored in casks for observation of their stability. The changes in soluble nitrogen and soluble phosphorus content that occurred in the fermenting juice prior to centrifuging and in the ciders during storage were examined.

The factors responsible for the relative stability of the ciders after centrifuging were also studied by adding a number of different yeast nutrients, separately and in combination, to two of the ciders after storage, in an attempt to promote further fermentation.

†The terms total nitrogen, soluble nitrogen and nitrogen in suspension, and the corresponding terms for phosphorus, have been described previously (3).

*The minimum values for soluble nitrogen and soluble phosphorus and the times at which these were observed to occur, were also influenced by the autolysis of yeast.

The undisturbed fermentation of this Bramley juice, which was described in the previous paper, is to be regarded as the Control for these centrifuging experiments and frequent reference to it will be necessary.

EXPERIMENTAL.

The milling and pressing of the fruit and the bulking of the juice were briefly described in the previous paper (3). Care was taken to ensure that the bulked juice was thoroughly mixed before it was distributed between the eight casks. All casks were thoroughly cleaned; they were treated with 2% SO_2 solution, steamed for 30 minutes, cooled and allowed to drain before filling with fresh juice.

Part of the bulked juice was distributed uniformly into six hogsheads, each approx. 54 gal., and one 30 gal. cask (there was not sufficient juice to use hogsheads throughout).

While this was being done, the remainder of the bulked juice, approximately 40 gals., was pumped through the Sharples centrifuge (16,000 revs./min. and 80 gal./hour) collected into a Prodorglas-lined tank and filled into an eighth cask (Cask No. 2, capacity 30 gal.); this was the only cask to receive centrifuged fresh juice.

Three one-gallon jars of juice were then withdrawn from each of the eight casks and set aside as reserve material for topping up the casks when required. The casks themselves were finally re-filled with the bulked juice; Cask 2 was refilled with centrifuged juice.

All casks and jars were kept in the cider-house and fermentation was allowed to proceed in the normal way. When the "heads" had ceased to form, all casks and jars were cleaned and fitted with fermentation locks.

The fermenting juice in casks 3-8 was centrifuged (Sharples centrifuge; 16,000 revs./min., 80 gal./hour) at different stages of fermentation, i.e. after varying falls in specific gravity had occurred. After centrifuging, each of these ciders was collected in the Prodorglas-lined tank and pumped into a 30 gallon cask for storage and observation of its stability. The juice in Cask 1 was not centrifuged at any stage and this undisturbed fermentation (3) is therefore to be regarded as the Control for the fermentations which were checked by centrifuging. The centrifuged fresh juice in Cask 2 was allowed to ferment to dryness unchecked. After ten weeks storage, the once-centrifuged cider in Cask 3 was centrifuged a second time and was then stored in an 18 gallon cask; this cider was the only one which was centrifuged more than once.

Samples were taken from each cask as follows: (a) prior to fermentation (fresh juice); (b) at suitable intervals during fermentation prior to centrifuging; (c) immediately after centrifuging; (d) at suitable intervals during storage of the centrifuged cider in the cask. These samples were examined for specific gravity and for sugar, nitrogen and phosphorus content; the analytical procedures used have been described in the previous paper.

RESULTS AND DISCUSSION.

Details of the composition and properties of the bulked juice have been given in the previous paper (3) and only certain aspects are repeated here:—Specific gravity $1.051\frac{1}{2}$; total reducing sugars after inversion 10.80 g./100 ml.; total nitrogen 27.9 mg.N/100 ml.; soluble nitrogen 26.2 mg.N/100 ml.; total phosphorus 5.75 mg.P/100 ml.; soluble phosphorus 5.55 mg.P/100 ml. This Bramley juice had a very high nitrogen content and fermented rapidly.

Uniformity of Juice in the Different Casks.

Samples of fresh juice from all casks were examined for specific gravity and for soluble nitrogen content. The results showed a high degree of uniformity; specific gravity varied by less than half-a-degree (1.051 to $1.051\frac{1}{2}$) and soluble nitrogen ranged from 25.8 to 26.7 mg.N/100 ml. The average soluble nitrogen content of the juice calculated from the individual values for the eight separate casks was 26.1 mg.N/100 ml. and was in close agreement with the independent determination on the sample of bulked juice (26.2 mg.N/100 ml., see above).

This uniformity was also reflected in the course of the fermentation in all casks except Cask 2 (which was centrifuged as fresh juice) and Cask 4; thus, on the 15th day when fermentation was proceeding very rapidly (approx. 4 degrees decrease in S.G. per day) the specific gravities in Casks 1, 3, 5, 6, 7 and 8 were all between $1.026\frac{1}{2}$ and $1.028\frac{1}{2}$. The course of the fermentation of the centrifuged fresh juice in Cask 2 was very different from that of the juice which was centrifuged during fermentation (Casks 3-8); for this reason it is described separately. The onset of fermentation of the juice in Cask 4 was delayed by about five days due to the accidental presence of residual SO_2 in the cask. (For some unexplained reason, the SO_2 used in cleaning this cask was not completely removed in the subsequent cleaning and washing). However, once fermentation of the juice in Cask 4 commenced it proceeded at a rate comparable with that in Cask 1.

The rates of decrease which occurred in the soluble nitrogen content of the juices during fermentation, which may be regarded as representative of rate of yeast* growth (1), were also uniform up to the times of centrifuging in Casks 3-8, with the possible exception of Cask 5. The soluble nitrogen content of each of these ciders at the time of centrifuging (see Table I) was comparable with that of the fermenting juice in Cask 1 at the corresponding specific gravity (3).

The Fermentation of Centrifuged Fresh Juice (Cask 2).

The course of the fermentation of the juice in Cask 2, which had been centrifuged shortly after milling and pressing and before fermentation commenced, was very different from that observed in Casks 3 to 8. The fermentation was allowed to proceed to completion without further centrifuging and the course of fermentation was somewhat similar to that of the undisturbed fermentation (3) of the juice in Cask 1; there were, however, certain important differences which are summarised below. The changes in nitrogen content during fermentation are illustrated in the graph (Fig. 1); the scales employed are the same as those in Fig. 2 of the previous paper (3) for the fermentation in Cask 1, with which this graph should be compared.

The main features of the undisturbed fermentation of the centrifuged fresh juice in Cask 2 (and differences from Cask 1) were as follows:—

(a). The onset of fermentation was delayed until approximately the 10th day (c.f. in Cask 1, the 5th day).

(b). When fermentation commenced, it proceeded at a uniform rate which was rather slower than that of the juice in Cask 1. The average decrease in specific gravity was only 1.9 degrees per day between the 15th and 35th days whereas the fermentation in Cask 1, over a similar S.G. range, averaged 2.4 degrees S.G. per day between the 10th and 25th days.

*Microscopic examination showed that very few bacteria were present in the fermenting juice.

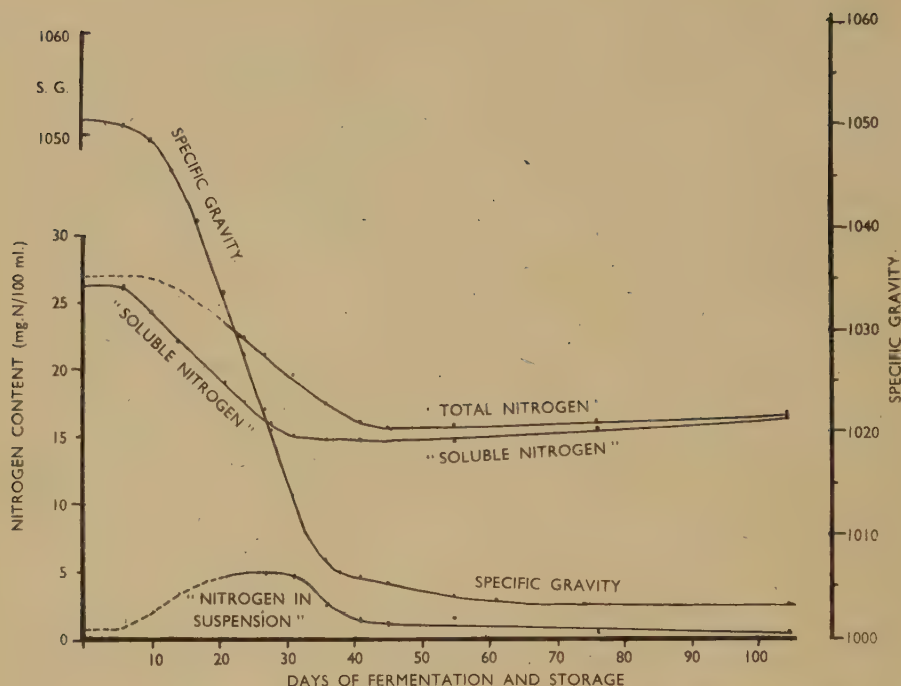


Fig. 1. Changes in Nitrogen Content during Fermentation of Centrifuged Fresh Juice and during Subsequent Storage.

(c). Utilisation of soluble nitrogen was much slower and took place at a uniform rate of 0.46 mg.N/100 ml. per day between the 6th and 28th days (c.f. in Cask 1—a uniform rate of 1.36 mg.N/100 ml. per day between the 6th and 16th days).

(d). The minimum soluble nitrogen of the fermenting juice in Cask 2 (14.5 mg.N/100 ml.) was considerably greater than that observed in Cask 1 (8.7 mg.N/100 ml.).

(e). The total nitrogen of the centrifuged fresh juice was 26.9 mg.N/100 ml., soluble nitrogen 26.3 mg.N/100 ml., and, by difference, nitrogen in suspension was 0.6 mg.N/100 ml. No further samples were taken for total nitrogen until the 21st day and the course of the increase in nitrogen in suspension over this period is therefore not certain. Subsequent determinations of total nitrogen and soluble nitrogen, however, show that nitrogen in suspension remained approximately constant during ten days (20th to 30th days); this is in marked contrast to the sharp peak observed for nitrogen in suspension at the 16th day in Cask 1.

The delay in the onset of fermentation in this centrifuged juice, compared with that in the Control juice (Cask 1), was probably largely due to the removal of part of the yeast population of the fresh juice by the centrifuge. The yeasts remaining in the juice were, however, able to grow and to ferment the juice to dryness, although the rate of fermentation was rather slower than that in Cask 1. This slower rate of fermentation may possibly have been due to a different balance of micro-flora in the fresh juice, resulting from the preferential removal by the centrifuge of yeasts of larger cell-size (4), but it could also be explained if it could be shown that less yeast growth took place in Cask 2.

In fact, the slower rate of utilisation of soluble nitrogen and the greater value of the minimum soluble nitrogen of the fermenting juice in Cask 2 indicate that both the rate and the extent of yeast growth were reduced as a result of centrifuging the fresh juice. While the slower rate of yeast growth might be accounted for on the basis of qualitative differences in the micro-flora present in Casks 1 and 2, this would not necessarily account for the fact that centrifuging the fresh juice reduced the total amount of yeast growth which subsequently occurred in that juice. An alternative explanation of the effect of centrifuging the fresh juice, on the rate and extent of yeast growth is suggested in a later section when the influence of growth factors in relation to fermentation is considered.

The Effect of Centrifuging at Different Stages during Fermentation (Casks 3 to 8).

The specific gravity and composition of the fermenting juice in Casks 3 to 8 at the times of centrifuging and the changes that occurred in the resulting ciders during storage (at 5, 8, 16 and 24 weeks) are shown in Table I. Other determinations of specific gravity were made at more frequent intervals and the changes which occurred are illustrated in the graph (Fig. 2). For comparison, the course of the fermentation of the Control juice in Cask 1 is also shown in this graph.

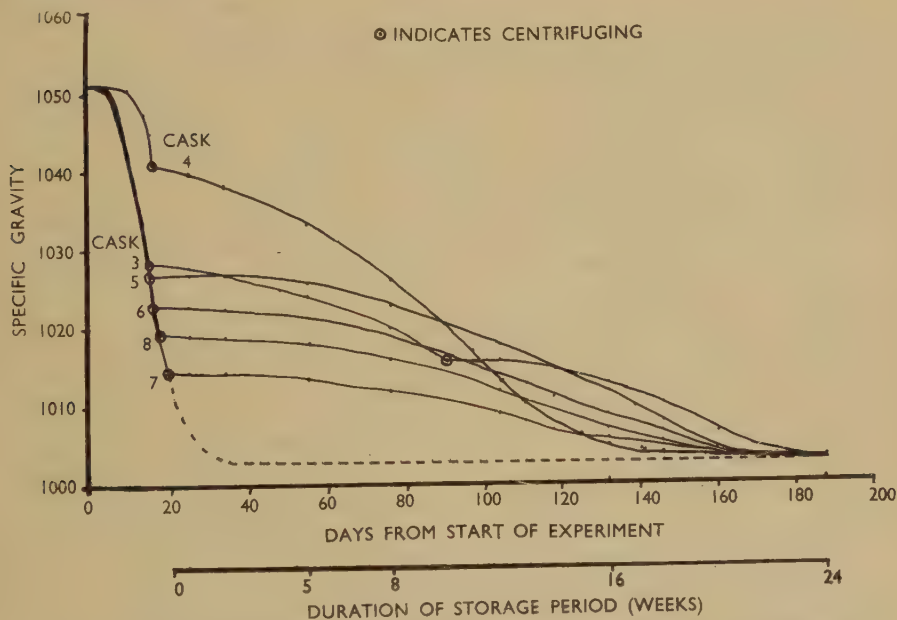


Fig. 2. Changes in Specific Gravity of the Fermenting Juice prior to Centrifuging and of the Resulting Ciders during Storage. The undisturbed fermentation of the Control (Cask 1) is shown by the dotted line.

Changes in Specific Gravity and Sugar Content.

(a) Prior to Centrifuging.

This Bramley juice had an original specific gravity $1,051\frac{1}{2}$ and total reducing sugar content 10.80 g./100 ml. After an initial lag-period of five days, the Control juice in Cask 1 fermented rapidly (3) and when fermentation was proceeding at its maximum rate, between the 14th and 16th days, the

TABLE I.

THE COMPOSITION OF CIDERS CENTRIFUGED AT DIFFERENT SPECIFIC GRAVITIES :
DATA AT TIME OF CENTRIFUGING AND AFTER DIFFERENT PERIODS OF STORAGE.
(Casks are arranged in decreasing order of specific gravity at time of centrifuging.
Cask 1 (not centrifuged) is included for comparison.)

Cask No.	Duration of Fermentation prior to Centrifuging	Composition of the Ciders					
		Constituents	At Time of Centrifuging	During Storage			
				At 5th week	At 8th week	At 16th week	At 24th week
4	16 days	Specific Gravity	1,041	1,033½	1,026	1,005	1,003½
		† Total Sugars	8.50	6.65	—	0.55	0.10
		* Soluble Nitrogen	16.2	13.6	11.8	10.3	11.7
		* Soluble Phosphorus	3.60	3.05	2.75	2.65	2.95
3	15 days	Specific Gravity	1,028½	1,024	1,020	(Centrifuged a second time at 10th week of storage).	
		Total Sugars	5.70	4.57	—		
		Soluble Nitrogen	12.0	10.0	9.2		
		Soluble Phosphorus	2.40	2.45	2.25		
	—	Specific Gravity	1,016	—	—	1,013	1,003
		Total Sugars	—	—	—	2.30	0.10
		Soluble Nitrogen	8.5	—	—	7.4	6.0
		Soluble Phosphorus	2.05	—	—	1.90	1.85
5	15 days	Specific Gravity	1,027	1,026	1,023	1,012	1,003
		Total Sugars	5.55	5.00	—	2.00	0.10
		Soluble Nitrogen	12.7	11.0	11.0	9.5	9.1
		Soluble Phosphorus	2.75	2.60	2.60	2.55	2.40
6	16 days	Specific Gravity	1,023	1,021½	1,019	1,009	1,003½
		Total Sugars	4.50	4.50	—	1.40	0.10
		Soluble Nitrogen	10.2	9.2	8.2	7.6	7.7
		Soluble Phosphorus	2.20	2.20	2.10	2.00	1.95
8	18 days	Specific Gravity	1,019½	1,018	1,016	1,007½	1,003
		Total Sugars	3.65	3.65	—	0.95	0.10
		Soluble Nitrogen	9.8	8.4	8.0	7.2	7.5
		Soluble Phosphorus	2.15	2.00	1.85	1.80	1.80
7	20 days	Specific Gravity	1,014½	1,013½	1,012	1,006	1,003
		Total Sugars	2.70	2.75	—	0.80	0.10
		Soluble Nitrogen	9.6	9.1	8.9	8.3	7.9
		Soluble Phosphorus	2.20	2.25	2.20	2.15	2.20
1	—	Specific Gravity	Not Centrifuged	1,003	1,003	1,003	1,003
		Total Sugars		0.15	0.15	0.15	0.10
		Soluble Nitrogen		10.3	10.8	11.6	12.7
		Soluble Phosphorus		3.35	3.60	3.70	3.75

† Total Sugars were determined as total reducing sugars after inversion with invertase and are expressed as invert-sugar g./100 ml.

* Soluble Nitrogen and Soluble Phosphorus are expressed as mg./100 ml.

NOTE :—Centrifuging took place between December 28th (Casks 3 and 5), and January 2nd (Cask 7). The period of storage is regarded as commencing on January 2nd, 1950.

specific gravity decreased by 4.5 degrees per day, i.e., sugar was being fermented at a rate of approximately 1 g./100 ml. per day. As stated earlier, the onset and the course of fermentation of the juice in each of the Casks 3 to 8 (except Cask 4) were very uniform and comparable with the fermentation of the juice in Cask 1. In the juice in Cask 4, which was contaminated with sulphur dioxide, onset of fermentation was delayed but, once fermentation commenced, it proceeded at a rate comparable with that in Cask 1.

(b) After Centrifuging.

The check to fermentation which was obtained by centrifuging the fermenting juices was very marked in comparison with the rapidity of the fermentation prior to centrifuging (Fig. 2). Nevertheless, all centrifuged ciders ultimately fermented to dryness (S.G. 1.003; total reducing sugars 0.10 g./100 ml. at the 24th week); the undisturbed fermentation of the juice in Cask 1 reached dryness in only 5 weeks. Although none of the centrifuged ciders was completely stable, the degree of control of fermentation achieved in all casks, except 4 and 3, was adequate from the practical point of view and would have allowed the cider maker to treat these ciders further, e.g. by filtration, to assist retention of the natural sugars.

The degree of control of fermentation achieved by the different centrifuging treatments is illustrated in the graph (Fig. 2) and is expressed in Table II in terms of decrease in specific gravity during the first 8 weeks of storage in the cask. Table II also includes laboratory determinations of the relative stability of the ciders made by incubating (25° C.) 100 ml. volumes of the centrifuged cider and determining the loss in weight, due to evolution of CO₂, during 30 days. A loss of 1 g./100 ml. corresponds to a decrease of approximately 10 degrees of specific gravity, and to the disappearance of approximately 2 g. sugar per 100 ml. cider.

TABLE II.
RELATIVE STABILITY OF THE CIDERS OBTAINED BY
CENTRIFUGING THE FERMENTING JUICES AT DIFFERENT SPECIFIC GRAVITIES.

Cask No.	Decrease in Specific Gravity prior to Centrifuging (degrees)	Specific Gravity at Time of Centrifuging	Decrease in Specific Gravity during 8 weeks Storage in Cask (degrees)	Loss in Weight of Cider incubated at 25° C. during 30 days (g./100 mls.)*
4	10	1.041	15	2.53
3	22½	1.028½	8½	1.50
5	24	1.027	4	1.07
6	28	1.023	4	0.86
8	31½	1.019½	3½	0.79
7	36½	1.014½	2½	0.49

*Laboratory determination on 100 ml. volumes of cider.

It will be seen that the relative stabilities of these ciders, both during storage in the cask and during incubation at 25° C., are related to the decrease in specific gravity which occurred prior to centrifuging. Thus the cider in Cask 4 which was centrifuged after a decrease of only 10 degrees specific gravity (at S.G. 1.041) lost 15 degrees of gravity in 8 weeks and, as is shown in Fig. 2, was the first to reach dryness. The cider in Cask 3 which was centrifuged after a greater decrease (22½ degrees) of specific gravity was rather more stable, but the subsequent rate of decrease in specific gravity was still quite rapid (8½ degrees S.G. in 8 weeks). In an attempt to prevent further loss of specific gravity, this cider was centrifuged a second time, at the 10th week

of the storage period when the S.G. was 1,016. As shown in the graph, the resulting check to fermentation was only temporary and lasted about 3 weeks, after which the rate of decrease of specific gravity was comparable with that in the other casks.

The ciders in Casks 5, 6, 8 and 7 did not differ greatly in stability, although that in Cask 7 was the most stable. All these ciders were more stable than those in Casks 4 and 3.

Changes in Nitrogen Content.

(a) Soluble Nitrogen Content of the Ciders at Time of Centrifuging.

As stated earlier, the soluble nitrogen content of each cider at time of centrifuging was similar to the soluble nitrogen content of the fermenting juice in Cask 1 (Control) at the corresponding specific gravity. In Cask 1, utilisation of soluble nitrogen was rapid until the specific gravity had fallen to 1,022; subsequently, soluble nitrogen was utilised more slowly until the specific gravity was in the region of 1,008 when utilisation ceased. The ciders in Casks 4, 3 and 5 were centrifuged while utilisation of soluble nitrogen was still actively progressing and consequently the soluble nitrogen contents of these ciders were relatively high. On the other hand, the ciders in Casks 6, 8 and 7 were each centrifuged during the period of slower utilisation of soluble nitrogen. Thus, all these ciders (Casks 3-8) were centrifuged before growth of yeast, as measured by utilisation of soluble nitrogen, had ceased.

All these ciders fermented slowly during storage but in general a high soluble nitrogen at time of centrifuging was associated with a rather greater tendency to ferment. Thus, at the time of centrifuging, the soluble nitrogen contents of the ciders in Casks 4 and 3 were greater than those of the ciders in Casks 6, 8 and 7; the former fermented rather more rapidly during storage. The cider in Cask 5, however, which was much more stable than that in Cask 3, was exceptional and had a soluble nitrogen content at the time of centrifuging slightly greater than that of the cider in Cask 3.

(b) Changes in the Soluble Nitrogen Content of the Centrifuged Ciders during Storage.

The Control cider in Cask 1, which was not centrifuged but was allowed to stand on its lees, showed a considerable increase in soluble nitrogen content during storage due to autolysis of yeast (3). In contrast, the soluble nitrogen content of the centrifuged ciders (Casks 3 to 8), in general decreased progressively during storage; these changes are shown graphically in Fig. 3 together with the change in soluble nitrogen content of the cider in Cask 1.

The greatest decrease in soluble nitrogen was observed in the cider in Cask 4 (16.2 to 10.3 mg.N/100 ml.), this decrease taking place during the first 16 weeks. The soluble nitrogen content of this cider subsequently increased and was 11.7 mg.N/100 ml. at the 24th week of storage; this increase may be attributed to autolysis of the yeast which had grown in the cider after centrifuging.

The soluble nitrogen content of the cider in Cask 3 decreased from 12.1 to 8.5 mg.N/100 ml. during the first 10 weeks of storage. This decrease was less than that observed in Cask 4 but was greater than that in any of the other casks. The second centrifuging of the cider in Cask 3 did not prevent further fermentation and the yeast growth associated with this fermentation was sufficient to reduce the soluble nitrogen content to 6.0 mg.N/100 ml. at the

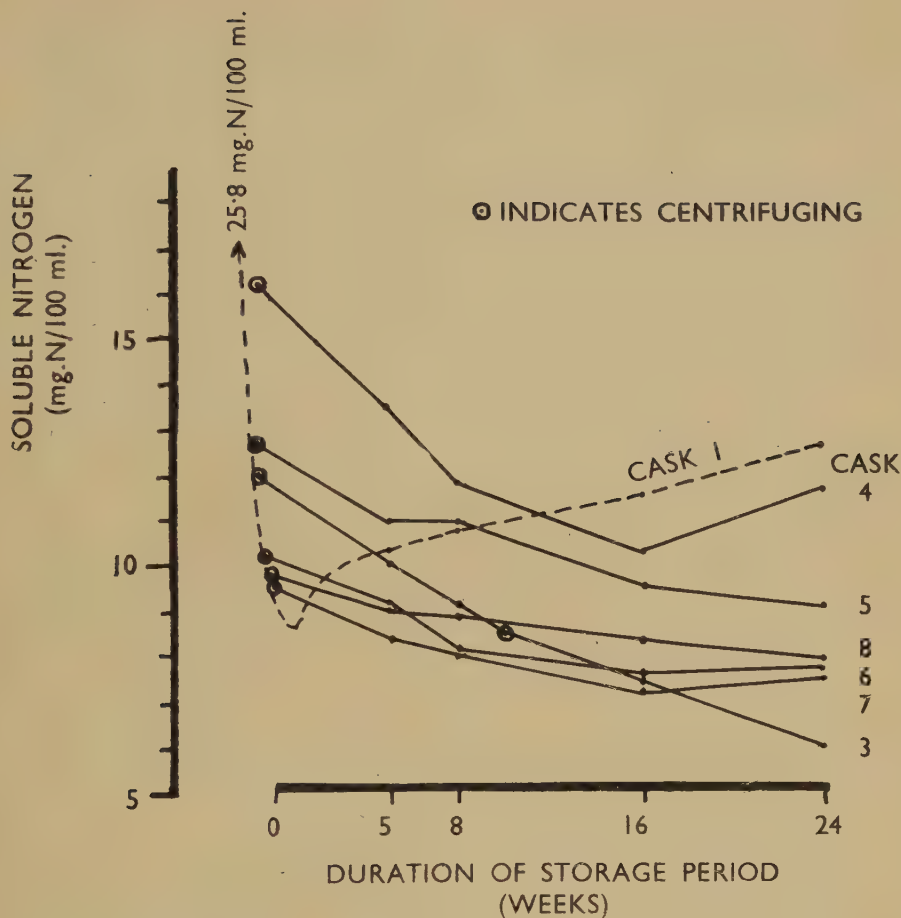


Fig. 3. Changes in Soluble Nitrogen Content of Centrifuged Ciders during Storage.

N.B. (1). The scale for soluble nitrogen is different from that used in Fig. 1 and has been magnified in order to separate the curves for the individual casks.

(2). The changes which occurred during the undisturbed fermentation (Cask 1) are indicated by the dotted line.

24th week of storage. This was the lowest soluble nitrogen content recorded in any of these ciders.

The decreases in soluble nitrogen which occurred in the ciders in Casks 5, 6, 8 and 7 during 24 weeks of storage were considerably less than those observed in Casks 4 and 3. Thus, in general, the decrease in soluble nitrogen content of the ciders during storage could be associated with the decrease in specific gravity during this time.

Changes in Phosphorus Content.

In the undisturbed fermentation in Cask 1 (Ref. 3) changes in the phosphorus content (total, soluble and in suspension) of the fermenting juice were closely similar to the corresponding changes in nitrogen content, during the time when yeast was actively growing, i.e., before autolysis occurred. This could be explained by the fact that both soluble nitrogen and soluble

phosphorus were utilised by the yeast, and the changes which occurred in these constituents, were due to growth of yeast. For the same reason, there was a close similarity between changes in soluble nitrogen and soluble phosphorus content of the fermenting juices (up to the times of centrifuging) and of the ciders during storage, in Casks 3 to 8. In consequence the statements made regarding the changes in nitrogen content, in general, also apply to phosphorus and the changes in phosphorus content are therefore described very briefly. Thus :—

(a). At times of centrifuging, the soluble phosphorus content of the fermenting juice in each cask, with the exception of Cask 5, was similar to that of the fermenting juice in Cask 1 at the corresponding specific gravity. The explanation of the anomalous behaviour of Cask 5, which was also noted in its soluble nitrogen content, is not known.

(b). The fermenting juices in Casks 4, 3 and 5 were centrifuged while utilisation of soluble phosphorus was still actively progressing; their soluble phosphorus contents were therefore relatively high. On the other hand, utilisation of soluble phosphorus had almost ceased when the fermenting juices in Casks 6, 8 and 7 were centrifuged and the soluble phosphorus contents of these ciders were relatively low.

(c). The soluble phosphorus content of each of these ciders, in general, decreased during storage (in contrast to the marked increase, due to autolysis of yeast, observed during storage of the cider in Cask 1); the greatest decrease was observed for Cask 4, up to the 16th week of storage and this was followed by an increase up to the 24th week, presumably due to autolysis.

(d). In general, decrease in soluble phosphorus content of these ciders during storage, could be associated with decrease in specific gravity; thus the ciders in Casks 4 and 3 showed greater decreases in soluble phosphorus content than those in Casks 5 to 8.

The Addition of Yeast Nutrients to the Centrifuged Ciders after Storage.

As stated in the Introduction, experiments were carried out to ascertain whether the relative stability of the ciders after centrifuging was due to a deficiency of some essential yeast nutrient. A number of different yeast nutrients were added, separately and in combination, to two of the ciders after storage in an attempt to promote further fermentation. It was unfortunately not possible to make the addition of these nutrients before the stored ciders had fermented to dryness and it was therefore necessary to add fermentable sugar and an inoculum of yeast.* There were two separate experiments; the first involved the addition of a wide range of yeast nutrients and treatments were not replicated; in the second experiment, several of the nutrients which previously gave no response were omitted, while the effects of other nutrients were examined in triplicate. In both experiments two ciders were used, cider from Cask 3 (which had been centrifuged twice) and from Cask 7 (which had only received a single centrifuging). The same technique was employed in both experiments and is described below.

The dry ciders (approx. $2\frac{1}{2}$ litres of each) were sweetened by addition of one-tenth of their volume of approximately 50% (weight/volume) invert

*It was obviously preferable to avoid the use of added yeast and to study the effect of the added nutrients on the yeasts remaining in the centrifuged ciders. Such yeasts however, were relatively few in number and it was considered doubtful whether there would be sufficient viable organisms present in these stored ciders to give a marked response even when any nutritional deficiency was remedied.

sugar syrup, prepared from A.R. sucrose by inversion (HCl) and subsequent neutralisation (NaOH). The two ciders were similarly inoculated with a small quantity of actively fermenting yeast, obtained from another Bramley cider by centrifuging, and then filled into a series of 4 oz. medicine bottles (110 ml. capacity). Each bottle, except two which were used as Controls, contained a known amount of a single yeast nutrient or combination of nutrients. Finally, each bottle was closed with a fermentation lock, weighed and kept at room temperature for yeast growth and fermentation to take place. The different rates at which these ciders fermented were measured by determining the loss in weight of each bottle, due to evolution of CO_2 , at suitable intervals.

In the first experiment the following yeast nutrients were added, singly and in different combinations: *Nitrogen* (as NH_4Cl); *Phosphorus* (as KH_2PO_4); *Magnesium* (as MgCl_2); *Sulphur* (as K_2SO_4). *Trace elements* were added as a composite solution (supplied by Dr. D. J. D. Nicholas, Plant Nutrition Section) containing the micro-nutrients iron, manganese, copper, zinc and molybdenum. *Growth factors* (seven members of the vitamin B complex) were added in the form of a solution such that 1 ml. in 110 ml. of cider provided each of the growth factors at approximately the concentration recommended by Hopkins and Pennington (6) for growth of yeast.

Pyridoxin	1.0	$\mu\text{g.}/\text{ml. cider}$
Aneurin hydrochloride	0.25	" "
Inositol	25.0	" "
d. Ca pantothenate	2.5	" "
Nicotinic acid	2.5	" "
Biotin	0.0075	" "
p. Aminobenzoic acid	2.0	" "

The results of this experiment are not given here in full since they were essentially preliminary in nature; the treatments were designed to cover a wide range of combinations of nutrients and individual treatments were not replicated. The results showed clearly that in both these ciders, further fermentation was limited by a deficiency of one or more of the growth factors. No other nutrient, or combination of nutrients which did not include growth factors, gave a response comparable with that obtained in the treatments where growth factors were added.

The results of the second experiment, in which the effects of nitrogen, phosphorus, magnesium and growth factors were investigated separately and in combination, are shown in Table III; they are expressed in terms of loss in weight due to evolution of CO_2 . The table also includes the decrease in specific gravity which occurred after 17 days, calculated from the loss in weight (a loss of 0.11 gm./110 ml. is equivalent to a decrease of one degree S.G.).

In confirmation of the first experiment, these results show that the fermentation of the ciders in these small scale tests was limited by deficiency of one or more of the growth factors. Statistical analysis of the data (17 days) in Table III shows that the effect of added growth factors was highly significant at the level $P = 0.01$, for each of these ciders (Casks 3 and 7).

The cider from Cask 3 also showed certain differences due to added mineral nutrients (N, P, Mg) in the presence of added growth factors (G.F.), thus: (a) the increased response due to the addition of (N + P + Mg) in the presence of G.F. was highly significant, at the level $P = 0.01$; (b) the difference in response between (N + G.F.) and (N + P + Mg + G.F.) was also significant, at the level $P = 0.05$; (c) the difference in response between

TABLE III.
EFFECT OF ADDITION OF GROWTH FACTORS AND OTHER YEAST
NUTRIENTS ON THE FERMENTATION OF CENTRIFUGED CIDERS.

Nutrients Added	Loss in Weight due to evolution of CO ₂ (g./110 ml.)				Calculated* Loss in Specific Gravity (degrees)	
	After 7 days		After 17 days		After 17 days	
	Cask 3	Cask 7	Cask 3	Cask 7	Cask 3	Cask 7
No Additions (Control)	0.02 0.02 0.03	0.00 0.10 0.03	0.21 0.22 0.26	0.14 0.30 0.19	2	2
Nitrogen alone	0.04	0.02	0.29	0.21	2½	2
Phosphorus alone	0.00	0.08	0.23	0.24	2	2
Magnesium alone	0.02	0.07	0.20	0.17	2	1½
Nitrogen, Phosphorus and Magnesium	0.14 0.00 0.06	0.02 0.11 0.12	0.24 0.17 0.27	0.14 0.39 0.36	2	2½
Growth Factors alone	0.04 0.10 0.06	0.49 0.76 0.55	0.63 0.89 0.76	1.35 1.64 1.29	7	13
Nitrogen and Growth Factors ..	0.07 0.06 0.21	0.60 0.48 0.56	0.88 0.78 1.47	1.37 1.05 1.24	9½	11
Nitrogen, Phosphorus, Magnesium and Growth Factors	0.26 0.32 0.16	0.46 0.40 0.56	1.55 1.75 1.08	1.09 1.06 1.31	13½	10½

The amounts of nutrients added in the above treatments were as follows :—
Nitrogen, 10 mg.N/100 ml. ; Phosphorus, 5 mg.P/100 ml. ; Magnesium, 5 mg. Mg/
100 ml. ; Growth Factors, 1 ml. of composite solution described in text.

* A loss of 0.11 g.m. CO₂ per 110ml. cider corresponds to a loss of one degree of
specific gravity. Where triplicate determinations were made, only the average
loss of specific gravity is quoted.

G.F. alone and (N + G.F.), on the other hand, was not significant. In the
cider from Cask 7, however, the effects of addition of mineral nutrients in the
presence of growth factors were not significantly different from the effect
observed with growth factors alone.

Thus it appears that, while in both ciders fermentation was limited by
deficiency of growth factors, in the cider from Cask 3 one, or more, of the
elements, nitrogen, phosphorus and magnesium were present in suboptimal
amounts although not actually preventing fermentation when growth factors
were added. This difference between ciders from Casks 3 and 7 may possibly
be attributed to the fact that the cider from Cask 3 had been centrifuged twice
and the yeast growth which occurred after the second centrifuging had further
depleted the supplies of nitrogen, phosphorus and magnesium in the cider.

Two points must, however, be emphasised in connexion with the inter-
pretation of these results.

(a). The response measured in these experiments was the weight of CO₂
evolved and was a measure of the extent of fermentation. Although this was
probably related to the amount of yeast growth which occurred in the differently

treated ciders, yeast growth was not necessarily proportional to the observed extent of fermentation. Measurement of the fermentation resulting after the addition of different yeast nutrients does, however, indicate the probable nature of the nutritional deficiency responsible for the stability of the ciders.

(b). The yeast inocula used in these experiments were obtained from a naturally-fermenting Bramley juice, which was considered the most suitable available source of yeast for this purpose. These yeasts, however, were not those responsible for the primary fermentation of the ciders under test and furthermore the inocula would contain yeasts of large cell-size as well as the smaller yeasts which normally predominate in a centrifuged cider (4). Therefore, since different yeasts are known to vary in their growth factor requirements, the results of these small scale tests cannot be applied strictly to the behaviour of these centrifuged ciders. They can only be regarded as indicating that the degree of control of fermentation which was achieved by centrifuging was probably due to removal of one or more growth factors. In view of this fundamental limitation it was decided not to investigate the effects of individual growth factors added to these particular ciders. The part played by growth factors in the control of fermentation by the centrifuge method will be further examined in a later paper.

If this interpretation is correct and the relative stability of the ciders was in fact due to deficiency of growth factors, it is probable that the extent and possibly the rate of growth of yeast during the undisturbed fermentation (Cask 1) were determined by the amounts of growth factors present in the juice. The implications of this are discussed in the following section.

The centrifuging of the fresh juice (Cask 2) presents features of special interest. The slower and less complete utilisation of the "soluble nitrogen" of the centrifuged fresh juice in Cask 2, may possibly have been due to the removal of growth factors, absorbed on, or in the solid apple tissue separated from the juice by centrifuging. Ives *et al.* (7) have shown that, in some fruits and vegetables, up to 80% of certain of the growth factors present is associated with the solid tissue. Although they did not examine the apple in this respect, it is probable that at least part of the growth factor content of freshly-pressed apple juice is associated with the small particles of apple tissue and the "wild" yeasts present in suspension. Centrifuging the fresh juice would therefore partially remove some of the growth factors and yeast growth in that juice would be correspondingly reduced, provided other yeast nutrients were present in relative excess. This explanation is put forward as an alternative to that suggested in an earlier section based on the effect of centrifuging on the balance of micro-flora in the juice. Direct experimental evidence is required to distinguish between the two possible mechanisms.

Discussion of the Mechanism of the Control of Fermentation by the Centrifuge.

In the experiments described in this paper, the stability of the ciders obtained by centrifuging the fermenting juice at different specific gravities, was, as expected, dependent on the decrease in specific gravity which had occurred prior to centrifuging. This relationship, which is well known in cider-making practice, was noted by Charley (4 and 5) who suggested that the mechanism of the process may be related to the removal of nitrogenous substances from the fermenting juice as the result of the growth of yeast.

As stated in the Introduction, the present authors have previously

examined this problem from the point of view of the utilisation of the soluble nitrogen of the fermenting juice prior to centrifuging (2). In that paper, all the centrifuged ciders were relatively stable during storage although small differences in stability were observed. Since each of these ciders had in fact reached the minimum soluble nitrogen content before centrifuging, they each had approximately the same soluble nitrogen content. Consideration of the experimental data for the fermentation of that Bramley juice (Refs. 1 and 2) led to the tentative conclusion that stability could not be expected in a centrifuged cider unless the centrifuging had been carried out after the utilisation of the readily available soluble nitrogen was complete. It was also suggested that some other factor might be involved and that this factor might be phosphorus.

In the present investigation, care was taken in the planning of the experiment to ensure that the fermenting juice in the different casks would be centrifuged at such times that utilisation of soluble nitrogen would be incomplete in some, and complete in others. This object, however, could not be satisfactorily achieved owing to the very high nitrogen content of the fresh juice; thus, the specific gravity of the fermenting juice had fallen to a very low value (S.G. 1.008) before utilisation of soluble nitrogen ceased.

These centrifuged ciders were less stable than those previously examined (2) and also showed greater individual differences in degree of stability. These differences could be related to the decreases in specific gravity which occurred prior to centrifuging the fermenting juice and to the amounts of soluble nitrogen and soluble phosphorus utilised during that time. As stated in the Introduction, utilisation of soluble nitrogen and of soluble phosphorus can each be regarded as representing growth of yeast. Thus, the degree of stability of these ciders after centrifuging was apparently related directly to the extent of yeast growth which occurred prior to centrifuging. This statement has wider implications than that made provisionally in the earlier paper (2), namely "that effective control of the fermentation of a juice may be expected if the juice is centrifuged after utilisation of the readily available soluble nitrogen is complete."

In order to understand more fully the mechanism of the control of fermentation of cider by the centrifuge, it is necessary to consider the process of yeast growth and fermentation as it occurs in apple juice from a wider and more fundamental point of view than that of the utilisation of soluble nitrogen or soluble phosphorus. The course and extent of the growth of yeast in a fermenting juice depends, among other factors, on the adequacy of the supply of essential yeast nutrients in that juice. Such nutrients would include mineral salts, yeast assimilable nitrogen (e.g. amides, amino acids and simple peptides), fermentable sugars and growth factors. As growth of yeast and fermentation proceeds, the yeast nutrients are utilised and the amounts present in the juice decrease until finally one (or more) of these nutrients is reduced to such a low concentration that growth of yeast* becomes very slow or ceases. Thus, if a fermenting juice is centrifuged at different stages during its fermentation, i.e. at different specific gravities, the ciders obtained will contain decreasing amounts of residual yeast nutrients depending on the extent of yeast growth which occurred prior to centrifuging. The amounts

*It should be noted that *fermentation* does not normally cease at this stage but continues unchecked, at a rate dependent on the amount of yeast actually present, on the power of fermentation of that yeast and on other factors such as the amount of fermentable sugar left in solution, the alcohol content, pH etc.

of yeast nutrients remaining in a cider after centrifuging will determine the extent to which further growth of yeast can occur in that cider and will thus influence the rate at which the cider will ferment during storage. The power of fermentation of this yeast, however, is another factor involved and this aspect is considered in a later paragraph.

The nature of the yeast nutrient, or nutrients, which are deficient in a centrifuged cider and thus limit the amount of yeast growth that can occur, may vary according to the nature of the juice from which the cider was made and according to the individual nutritional requirements of the different yeasts present in the mixed microflora. Whatever the nature of these nutrients, if the fermenting juice is centrifuged after growth of yeast has ceased, the resulting cider, although it may contain fermentable sugar, will be unable to support further growth of the yeasts which pass through the centrifuge. Such a cider may be expected to remain relatively stable and retain its natural sugars during storage, although if a yeast of different nutritional requirements gained access to it, its stability would be uncertain. Thus, it is suggested that effective control of the fermentation of a juice may be expected if that juice is centrifuged after growth of yeast has ceased.

It is, however, realised that growth of yeast probably does not cease entirely when the soluble nitrogen (or soluble phosphorus) content of the juice reaches its observed minimum value. Changes in soluble nitrogen content are an expression of the balance between its utilisation for yeast growth, and its liberation due to autolysis of yeast. Therefore, just as some autolysis may be occurring while soluble nitrogen is still being utilised, so some degree of yeast growth may continue after the soluble nitrogen content of the juice has ceased to fall. Furthermore, it is probable that at least part of the soluble nitrogen, and soluble phosphorus, liberated by autolysis is utilised immediately for further growth of yeast and this growth would not therefore be detected analytically. It is not possible to state with certainty that the amount of yeast growth resulting from these causes would be relatively small, although this view is tentatively taken here. Nevertheless, a very slow and chemically undetectable growth of yeast, if occurring over a long period of time, may have an important influence on the stability of a centrifuged cider. This aspect of the problem can best be elucidated by microbiological observations and it is hoped to do this in later work.

The ciders examined in these experiments, however, were all centrifuged before growth of yeast had ceased and all fermented to dryness during storage (24 weeks). The decreases in specific gravity which occurred after centrifuging were accompanied by growth of yeast, as shown by the further decreases in the soluble nitrogen and soluble phosphorus contents of the ciders. However, the rates of decrease in specific gravity of these ciders during storage were slow, especially during the first few weeks. While this was probably largely due to the limited amount of yeast growth which occurred in these ciders, it is possible that the lack of some nutrient or other factor essential for fermentation as distinct from growth of yeast, may also have been concerned. The distinction between substances essential for growth of yeast and those which may affect the vigour (power of fermentation) of yeast, may lead to an elucidation of the mechanism of the control of fermentation by centrifuging. This aspect is further considered in connexion with the effect of growth factors on yeast growth.

The experiments described in this paper have shown that the relative stability of these centrifuged ciders was probably due to a deficiency of

growth factors, since the addition of these factors (a composite solution containing seven members of the vitamin B complex) restored the ability of the cider to undergo fermentation. This finding is of fundamental importance since it is known that the supply of growth factors in a fermentation medium not only affects the amount of yeast growth which can occur but also influences the power of fermentation of that yeast. Hopkins and Pennington (6) have examined three different yeasts in this respect. They cultured these yeasts in media deficient in certain growth factors of the vitamin B complex and showed that their power of fermentation (manometric determination on measured amounts of yeast) was markedly reduced. Although the yeasts, and the conditions under which they were examined, were different from those occurring in cider, their conclusions may have an important bearing on the mechanism of the control of fermentation of cider. Thus, it is reasonable to postulate that a fermenting juice becomes progressively more deficient in growth factors as growth of yeast proceeds and that the amounts of yeast which will be able to grow in the ciders centrifuged at progressively decreasing specific gravities may, therefore, progressively decrease. Furthermore, since the cider in which these yeasts are present is deficient in growth factors, any yeast which is able to grow in it may also have a reduced power of fermentation. If this reasoning is correct, it would provide a possible explanation of the differences in the stabilities of the Bramley ciders previously examined (2) which could not be explained on the basis of their residual soluble nitrogen contents. The influence of growth factor deficiency on the power of fermentation of yeast might also account for the inactivation of the yeasts present in centrifuged ciders which was reported by Barker and Grove (8). Hopkins and Pennington have also shown (*loc. cit.*) that the power of fermentation of yeast cells (*Saccharomyces carlsbergensis*) grown in media deficient in any one of the growth factors, biotin, pantothenic acid or pyridoxin, was not appreciably stimulated by addition of the deficient factor unless a source of available nitrogen was present. Such interrelations between growth factors and other nutrients may also influence the stability of centrifuged ciders.

Our present knowledge of the mechanism of the control of fermentation of cider by centrifuging may be summarized in the following way. Ciders which have been clarified by centrifuging will contain some yeasts and the degree of stability obtained will depend upon the numbers of such yeasts and on the nutritional value of the centrifuged cider in which these yeasts are present. A high degree of stability may be expected if the cider is centrifuged when yeast growth has exhausted the cider of some nutrient essential for growth of yeast or of some factor which affects the power of fermentation of these residual yeasts and their progeny. In a cider derived from a juice of low initial nitrogen content, it is possible that a simple deficiency of nitrogen might arise in the centrifuged cider although this has not yet been demonstrated with certainty. Evidence has been produced, however, that a deficiency of growth factors of the vitamin B complex can occur.

The work described in this paper, while contributing materially to the understanding of the control of the fermentation of a cider, has indicated the need for a systematic study of the nutritional requirements of the yeasts occurring in a fermenting apple juice. In particular, an investigation both biochemical and microbiological in nature is required on the influence of growth factors on the course of yeast growth and on the vigour of that yeast in bringing about fermentation, both in the fermenting juice and in the ultimate cider.

SUMMARY.

1. A Bramley juice was centrifuged at different stages during fermentation and the centrifuged ciders were stored in casks for observation of their stability. Although none of these ciders was completely stable, different degrees of stability were observed.
2. The relative stabilities of the centrifuged ciders were apparently related (a) to the decrease in specific gravity and (b) to the utilisation of soluble nitrogen and of soluble phosphorus, which occurred prior to centrifuging.
3. The utilisation of soluble nitrogen and of soluble phosphorus prior to centrifuging, has been interpreted as a measure of the extent of yeast growth which occurred during that time.
4. Therefore (par. 2 and 3), the degree of control of fermentation which was achieved by centrifuging was related to the extent of yeast growth which occurred prior to centrifuging. It is suggested that effective control of the fermentation of a juice may be expected if the juice is centrifuged after growth of yeast has ceased.
5. The centrifuged ciders, after the addition of sugar and a small inoculum of yeast, did not ferment appreciably. Various yeast nutrients were added in an attempt to promote fermentation and a deficiency of one or more components of the vitamin B complex was indicated.
6. The need for a systematic study of the nutritional requirements of the yeasts growing in a fermenting apple juice is demonstrated.

Acknowledgments.

The authors wish to thank Messrs. R. J. Hathway, J. H. Llewellyn and J. B. White for help in the Cider House and Miss Yvonne P. May, Mrs. Barbara A. Baker, Miss Jeanne H. Baker and Mr. R. A. Coggins for assistance with the analytical work.

REFERENCES.

1. CHALLINOR, S. W. and BURROUGHS, L. F., *Ann. Rept. Long Ashton Res. Stn.*, 1948, 182.
2. BURROUGHS, L. F. and CHALLINOR, S. W., *ibid.*, 1948, 207.
3. BURROUGHS, L. F. and CHALLINOR, S. W., *ibid.*, 1949, 115.
4. CHARLEY, V. L. S., *ibid.*, 1933, 145.
5. CHARLEY, V. L. S., *ibid.*, 1938, 174.
6. HOPKINS, R. H. and PENNINGTON, R. J., *J. Gen. Microbiol.*, 1950, **4**, 171.
7. IVES, M., POLLARD, A. E., ELVEHJEM, C. A. and STRONG, F. M., *J. Nutrition*, 1946, **31**, 347.
8. BARKER, B. T. P. and GROVE, O., *Ann. Rept. Long Ashton Res. Stn.*, 1930, 199.

AN INVESTIGATION ON THE YEAST FLORA OF KINGSTON BLACK CIDERS. PART II.

By B. T. P. BARKER.

An account of the purpose of this investigation and the results of the first phase of the work was published last year (1). Its primary objective was to obtain precise information on the yeast flora concerned in the conversion of apple juice into cider under conditions of natural fermentation. Beginning with an examination of the yeasts present in the expressed juice seven days after the milling and pressing of the fruit (Stage A) a period adequate to permit some multiplication of the yeasts in question a second examination was made about three weeks later when the primary fermentation was at its peak (Stage B). This was followed by a final examination about ten months later, after the ciders had been filtered prior to bottling towards the end of active fermentation and had matured subsequently in bottle until nearly one year old from the date of milling and pressing of the fruit (Stage C). In this way it was possible to determine the character of the microflora at three of the most distinctive phases in the "fermentation" life of the cider.

Six ciders, each made from Kingston Black apples obtained from different centres—Budlake, Corse Lawn, Edgborough, Martock, Pendock and Staplegrove—were selected for examination in the above manner. In every instance the liquor was kept throughout its life at the ordinary air temperature in the cider-house at the Research Station. Details as to the dates of processing operations, the source of each sample of fruit, the characters of the respective juices as regards specific gravity, acid and tannin contents and rate of fermentation were given in Part I of this paper. This also contained in each instance the results of the examination of the yeast flora of the "freshly expressed" juice, (Stage A).

In Part II, now given, the corresponding results for the other two phases of the "fermentation" life, B and C, are described and the completed picture shown by the investigation is reviewed.

The Yeast Flora at the Peak of Active Fermentation. (Stage B).

The total number of isolates at this stage were 42, the numbers from the individual centres being Budlake 8, Corse Lawn 8, Edgborough 8, Martock 6, Pendock 8 and Staplegrove 4.

On plate cultures of the Budlake and Staplegrove ciders a few stray colonies of "pink" yeasts of the genus *Rhodotorula* were observed. These have not been taken into account in the above summary of isolates, being apparently chance airborne infections of the ciders in question or the plate cultures during preparation.

As with the A stage yeasts, each of the cultures obtained from the B and C stages was examined separately in respect of the following characters:— (a) appearance of its colonies on beer-wort gelatine plate and streak cultures; (b) the character of the vegetation in those cultures; (c) maximum temperatures of growth on beer-wort agar streaks; (d) action on glucose, fructose, maltose and lactose as regards fermentation; (e) ability to form spores on

potato and carrot cubes and porous porcelain (at ordinary room temperature and 25° C.), as well as in streak cultures on beer-wort gelatine.

As regards these isolates, the foregoing tests indicated that three of the Corse Lawn group corresponded so closely that they must be classed as being the same yeast. A pair of duplicates was found among the Edgborough isolates and one set of triplicates and one pair also in the Pendock set. Thus reduced, the number of distinct yeasts obtained from these centres becomes 6 for Corse Lawn, 7 for Edgborough and 5 for Pendock.

Comparison of the results thus obtained for the individual yeasts from each of the six centres at the three "fermentation" stages enables them to be grouped into 12 classes. The class character distinctions in general for Classes 1-11 are the same as those already given in Part I for the yeasts of the A stage, but in view of some of the results for the B and C stage yeasts it has been found desirable to sub-divide Classes 2 and 11. For a similar reason the addition of two new Classes 12 and 13 has been required. As thus modified, the class descriptions now stand as follows :—

Spore-forming.

Class 1. Cells medium to large, ovoid and sausage-shaped (young cultures); maximum growth limit about 34° C.; spore-bearing cells in most cases very numerous; ferments glucose, fructose, sucrose and maltose.

Class 2. *Zygosaccharomyces types.*

a. Cells medium, round-ovoid, very uniform; maximum growth limit 32-5° C.; spore formation preceded by cell conjugation; ferments glucose, fructose, sucrose and maltose.

b. Cells small to medium spherical and ovoid; maximum growth limit 34-35° C.; cell conjugation followed sometimes by spore formation; ferments glucose, fructose, sucrose and maltose.

c. Cells, maximum growth limit and fermentation as in *b*; cell conjugation more or less frequent, but no spore formation observed.

d. Cells, maximum growth limit, conjugation and spore formation as *b*; ferments glucose, fructose and sucrose, but not maltose.

e. Cells, maximum growth limit, fermentation and cell conjugation as *a*; spore formation in both conjugated and non-conjugated cells.

f. Cells of same general form and size as *b*, but with a much larger proportion of very small cells; maximum growth limit below 35° C.; fermentation as *b*; cell-conjugation with and without spore formation.

g. Cells small spherical; maximum growth limit 34-35° C.; ferments glucose, sucrose, fructose and maltose; spore fermentation following conjugation.

Non-spore-forming.

Class 3. Cells medium, roundish-ovoid, very uniform; maximum growth below 30° C.; ferments glucose, fructose and sucrose.

Class 4. Cells medium, ovoid, uniform with conspicuous vacuoles; maximum growth limit 32-5° C.; ferments glucose and fructose, forming a film on the surface of solutions of the latter.

- Class 5. Cells small to medium, round and ovoid ; maximum growth limit 38–41° C.; ferments glucose and fructose ; liquifies gelatine media.
- Class 6. Cells medium, sausage-shaped and very marked tendency to mycelioid arrangement ; maximum growth limit below 30° C.; does not ferment any of the sugars tested, but forms a film on fructose solutions.
- Class 7. Cells medium, ovoid, very uniform ; maximum growth limit about 42° C ; does not ferment any of the tested sugars, but forms a film on maltose and lactose solutions.
- Class 8. Cells small, ovoid, uniform ; maximum growth limit 38–40° C ; does not ferment any of the tested sugars ; liquifies gelatine media.
- Class 9. Cells small, ovoid, very uniform ; maximum growth limit 25–27° C.; doubtful slight fermentation of glucose and fructose.
- Class 10. Cells small, spherical and ovoid ; maximum growth limit 30–32° C.; does not ferment any of the tested sugars.
- Class 11. *Apiculatus* types.
- a. *Apiculatus* type ; small or medium lemon-shaped cells, budding at each end ; asporogenous ; ferments glucose and fructose ; maximum growth limit over 35° C.
 - b. *Apiculatus* type ; as a, but maximum growth limit about 30° C.
 - c. *Ludvigii* type ; medium to large lemon-shaped cells, budding at each end ; spore formation irregular and infrequent ; maximum growth limit over 35° C.

Slow-growing forms.

- Class 12. Cells more varied in shape than those of Class 11, but lemon-shaped forms are common ; asporogenous ; growth on solid media very slow ; maximum growth limits above 35° C.; ferments glucose, fructose, sucrose and maltose.

Class 1, as in the case of the “onset of active fermentation” stage is the most numerous and includes isolates from all centres. The cultures number 16 in all. Again, as in stage A, three appear to be identical and originate from the ciders of the same three centres—Budlake, Corse Lawn and Martock—as before. A fourth culture, practically indistinguishable from the preceding, appeared in the Edgborough group, although the occurrence of this form was not observed at stage A: two other forms—both distinct and found at stage A—were yielded by this cider. One of the latter two also occurred in the Corse Lawn group, although not found in that cider at stage A. Another possible duplication was noted, in which one of the yeasts of the Pendock group of this class and one of the Staplegrave were concerned. All the other cultures falling in Class 1 appeared to be slightly different from each other and from the above-mentioned forms. Thus the class comprises 11 yeasts including one appearing in the ciders of three centres, and two others duplicated at two centres in each instance. The distribution of the total number of cultures between the individual centres was as follows:—Budlake 3, Corse Lawn 3, Edgborough 3, Martock 2, Pendock 3 and Staplegrave 2.

Taking into account the vigorous fermentation character of these yeasts and the fact that the respective forms—with one exception—have persisted in considerable numbers to the peak of the fermentation period from its incipient stage, the importance of this class in cider-making under conditions of natural fermentation must be outstanding.

Class 2, which was represented at stage A by one variety of yeast in one only of the ciders, has increased its numbers threefold, the yeast in question having survived in the Corse Lawn cider in which it originally appeared and also having developed in those from the Budlake and Martock centres. The possible significance of this increase will not be discussed at this point, as this can be done more appropriately after the stage C results have been recorded.

Class 3 has shown little change from stage A. The two earlier samples, from Budlake and Edgborough ciders respectively, have reappeared again in those ciders and a similar form has now appeared in the Martock cider. In view of its persistence in the two earlier cases the Class may not be negligible as a factor in cider fermentations, but it hardly appears of top-grade importance.

Class 4 has provided no representatives at this stage, the two previous examples not having reappeared.

Class 5, which furnished 4 cultures at stage A—two from the Staple-grove cider and one each from those from Pendock and Edgborough—has now shown a reduction to three. The culture from Pendock has persisted and new appearances have occurred in the Budlake and Martock ciders. The two Staple-grove examples have not reappeared. Hence it seems likely that this class is of not more than secondary importance in cider fermentation.

Class 6, with its two representatives at stage A has now lost these and no further forms have developed.

Class 7, with a single culture from Edgborough at stage A, has retained it without addition.

Class 8, with a single culture from Corse Lawn at stage A has given a result similar to Class 7.

Classes 9 and 10 each included one culture only—from Martock and Budlake respectively—at stage A. In both cases these have disappeared and no new additions have occurred.

Class 11, in which are included the yeasts with lemon-shaped cells and terminal budding—the very common *apiculatus* forms and the less frequent *Saccharomycodes Ludwigii* types—has furnished results which suggest that its members may have a considerably more important role in cider production than has hitherto been attributed to them. The consideration of this point can be more appropriately deferred to the General Discussion which follows after the results for stage C have been given.

Each of the six ciders has one or more representatives in this Class, and all include at least one of the forms, *b*, with maximum growth temperature below 30° C.

The Yeast Flora of the Matured Ciders. (Stage C).

The ciders at this stage were examined in the following October, approximately eleven months after the fruit was milled and pressed. They were bottled direct from the filter, filtration taking place during the preceding January–March period at dates appropriate to the condition of the individual ciders. Their specific gravities at the time of filtration ranged from 1035 to 1043, these relatively high figures being due to the very high initial gravities of the freshly expressed juices and their slow rates of fermentation. During their life in bottle they were stored in a cool cellar.

Only five of the original six ciders were examined, a supply of the Pendock sample being no longer available at the time. The number of cultures isolated from the five centres totalled thirty-two, those from the respective

cider being as follows :—Budlake 6, Corse Lawn 5, Edgborough 10, Martock 4 and Staplegrove 7.

Two sets of duplicates were among the Corse Lawn isolates, two pairs of duplicates in the Edgborough set, one pair in the Martock set and two pairs in the Staplegrove set.

The numbers of individual types represented for the respective centres thus were Budlake 6, Corse Lawn 3, Edgborough 8, Martock 3 and Staplegrove 5.

The Class 1 yeasts still retained their earlier prominent position at this late stage in the life of the ciders. Six fairly distinct types were found and proved to be forms which had already been isolated in the earlier samplings. One of these was the form found in the freshly expressed juices from each of the three centres—Budlake, Corse Lawn and Martock—and isolated again from those three ciders at stage A. The Budlake cider also yielded one other member of this Class, which again had persisted from the initial stage. The Edgborough cider gave only one form, which similarly had been found at the two preceding stages. The Staplegrove cider provided two forms, also persistent from the start. From the Corse Lawn ciders, in addition to the duplicate of the Budlake and Martock samples above mentioned, a distinct form which had first shown itself in that sample at stage B was again isolated.

Class 2, which comprises yeasts characterised by cell conjugation prior to spore formation, increased strikingly the number of its members at stage C. After only one culture at the earliest stage and the appearance of this form in two other ciders at stage B the numbers jumped to 12 in the matured liquors. The definition of this class has been widened to allow the inclusion of certain yeasts which, while possessing the ability to conjugate have failed to produce spores as a sequence to that act under the usual spore formation tests; intermediate forms showing variable degrees of spore-forming ability are also included. A more doubtful type for classification is that in which—in old cultures especially—cells of irregular and abnormal shapes are produced; usually yeasts of this type are asporogenous and conjugation does not occur, but the cell forms referred to appear so akin in some cases to conjugating cells immediately before the fusion that they may have lost the power to conjugate or have not been subjected to conditions necessary for cell fusion. They may well be related genetically to the *Zygosaccharomyces* family and for present purposes are most conveniently included as a special group in this Class.

Under such classification twelve cultures have been placed in Class 2. They can be divided into six types. The commonest type is one which has appeared in the ciders from Budlake, Corse Lawn, Martock and Edgborough; this was also found in the first three centres at stage B and the Corse Lawn centre at stage A. Another type was common to the Budlake, Edgborough and Staplegrove ciders. A third form was duplicated in the Martock and Edgborough samples. Single representatives of the remaining types were formed in the ciders from Budlake and Edgborough (two forms).

Class 3 provided only one representative, this being in the Edgborough cider where it has also occurred at stages A and B.

Classes 4-9 were unrepresented in any of the ciders at this stage.

Class 10, contained one culture only, duplicated in isolations from the Staplegrove cider in which it first appeared at stage B.

Class 11, which in every cider at the earlier stages had been very conspicuous, had disappeared completely at this stage.

A very characteristic new Class, Class 12, made an appearance in four of

the five ciders—those of Budlake, Edgborough, Martock and Staplegrove. It contains only one form, an extremely slow-growing, small-celled non-sporing yeast on solid culture media with a maximum growth temperature limit as high as 42° C. The cell population was numerous in each instance. In spite of the slow growth rate—which possibly may account for the failure to isolate the organism at stages A and B—this yeast, by its capacity to ferment the four sugars named in the Class description, and its numerical prominence in the ciders in question, may be a factor affecting to some material extent the character of the matured product.

General Discussion.

The results of the detailed examination of the yeast flora of the six Kingston Black Ciders selected for the purpose at the three chosen critical phases of their fermentation life—the onset period of fermentation, the active fermentation period and the “maturity” period respectively—have been described in Part I and the preceding section of Part II of this paper. It now remains for them to be reviewed as a whole, to ascertain the nature of the general changes in the character of the microflora during the history of the ciders and the light thrown upon certain special questions, including the possible association of specific yeasts with ciders made from individual varieties of apples such as Kingston Black—the variety selected for the purpose of this investigation.

The associated work in connection therewith has involved the isolation of 118 yeasts and the examination of their distinguishing characters. With so large a number of isolates an exhaustive study of the latter was impracticable; hence the characters selected for determination were those likely to be most useful for the separation of the individual forms into distinctive classes and groups and the recognition of duplicates. Under such circumstances it was inevitable that the precise classification of very closely allied forms in certain cases should present some difficulty. However, so far as the main issues of the investigation are concerned, the conclusions which can be drawn from the results generally are so clear cut as a whole that they are unaffected by classification problems of that order.

As shown by the results in each of the ciders examined, the yeast cycle follows a definite course which is described in general terms in the succeeding paragraphs.

In a freshly expressed juice not subjected to any special form of treatment either while in the pomace before expression or afterwards—such, for example, as maceration or defecation—the number of different kinds of yeast present in sufficient quantity to appear in the plate-culture examination is of the order of from 5 to 8 at least. Invariably among these are one or more spore-forming kinds, akin in cell form to the *cerevisiae*, *ellipsoideus* or *pastorianus* types. Also at least one representative of the *apiculatus* group is regularly present. Normally the cells of the representatives of these groups are very numerous and constitute the major portion of the vegetation. In addition, usually from at least one to four other kinds of yeasts of various cell sizes and shapes occur; some of these can ferment glucose and fructose and some, in addition, sucrose. While usually they are not spore-forming kinds, in one of the juices examined, the Corse Lawn sample, a spore-forming conjugating yeast was present in very small numbers; this particular case is possibly specially significant in relation to the frequent and, possibly, regular appearance of *Zygosaccharomyces* forms in matured ciders. Otherwise the constituent

members of the yeast flora at the onset of fermentation, other than the spore-forming and *apiculatus* forms, can hardly be regarded as of major importance in cider fermentation; in occasional instances they may persist in the cider to the "maturity" stage, but more often they tend to disappear more or less early in its life. Such of them as can ferment the apple juice sugars doubtless contribute something towards the fermentation character, the extent presumably having some relation to their numerical proportion in the microflora; normally this is not high.

By the time the fermentation of the liquor has reached its peak period some changes of a minor character in the microflora are observable. The general tendency seems to be towards the more or less complete suppression of the non-sporing yeasts of the Classes 4 and 6-10. Class 3 yeasts can survive in appreciable quantity if their population in the newly expressed juice is fair or more; their ability to ferment glucose, fructose and sucrose may be of assistance towards their survival. (It is noteworthy that in one of the ciders examined a member of this Class was isolated at this stage, although its presence was not noticed in the newly expressed juice). Class 5 yeasts have given variable results; in the Pendock cider its representative among the four types isolated at stage A still survived in appreciable numbers at stage B, while that of the Edgborough cider and two forms in the Staplegrove cider had disappeared. On the other hand, in the Budlake and Martock ciders a single member of this Class was found at stage B, although not seen at stage A. The Class 1 spore-forming yeasts and the *apiculate* types of Class 10 continue to dominate the vegetation in all the ciders examined; no obvious signs of loss of place by the *apiculatus* were yet apparent in these three-week-old ciders, a result somewhat unexpected owing to their generally recognised sensitiveness to the presence of alcohol in quantities upwards of about 1 per cent, an amount which must have been reached in the actively fermenting liquors at that stage. Possibly the comparatively slow rates of fermentation of these ciders may have permitted a sufficient degree of aeration for the continuance of their multiplication. Most yeasts of the spore-forming types present at stage A reappear among the stage B isolations and some fresh types in some ciders become established; in a few cases forms present at stage A are missing at stage B. Yeasts of the *Zygosaccharomyces* Class begin to make their appearance more frequently; in the six ciders under examination single examples in the Budlake and Martock samples were found and the one form occurring in the Corse Lawn sample at stage A was again observed at stage B.

In the naturally sweet ciders matured in bottle for several months after the primary fermentation and subsequent filtration at a suitable specific gravity to permit natural conditioning in bottle, striking changes in the earlier microflora are apparent. The *apiculatus* yeasts have disappeared completely, as well as all the other yeasts of the Classes 3-10 with the following exception, in the ciders under consideration. One Class 3 yeast in the Edgborough cider which had been isolated at both the A and B stages still survived in that sample. In contrast, representatives of the Class 1 yeasts are found in all cases. Each of the ciders under investigation contained at least one of these spore-forming types, in three instances as many as three distinct types having survived: in all cases these survivors had been present throughout from the freshly expressed juice period, but no new forms had appeared. The characteristic features of this stage, however, are, firstly, an increase in the number of *Zygosaccharomyces* forms as well as the survival of such of these forms as had been noted in the earlier stages and, secondly, the appearance

of a very well defined new Class, consisting of small-celled *Torula* forms, very slow in their growth on solid culture media, with maximum growth temperatures well above 35° C., non-spore-forming, and fermenting dextrose, fructose, sucrose and maltose.

It should be noted that the features of the yeast flora in the matured bottled product correspond very closely with those found in an earlier investigation at this Station on the yeast flora of bottled ciders (2). In that instance five different ciders—three made from Sweet Alford apples and two from Kingston Black—were examined on lines similar to those adopted in the present case. The two main points of difference were that (a) in the former case the examination of the microflora was made only at the "matured" stage, the earlier microbiological history of the ciders being unknown, and (b) the ciders concerned had been bottled for a far longer period of time in three of the five cases—two years in two cases and one year in the third. Those differences, together with the fact that three of the ciders were made from the Sweet Alford variety, need to be taken into account in making comparison of the results of the two investigations and it is therefore all the more significant that in general they exhibit features so nearly in common. In comparing the results it should be mentioned that in both cases the major objectives were to ascertain (a) if there was any association of a particular yeast or yeasts with individual varieties of apples, and (b) if the fermentations in a given cider factory were determined mainly by a group of yeasts which might have a regular habitat there.

The present investigation has shown from the details already given that each cider has its own characteristic yeast flora and that, except in the case of *apiculatus* yeasts, there are few instances where the same yeast occurs in two or more of the ciders made at the factory on the same or two successive days. That applies not only to the initial stages of fermentation, but also to the period when active fermentation is at its peak, and later, after the liquor has matured in bottle. Nevertheless, at each stage of the yeast cycle, although the individual yeasts differ, there is a well-defined type of microflora characteristic of the respective stages in the life of all the ciders, and the predominant yeasts are clearly closely allied forms.

The earlier investigations, as in the case of the present one, showed that in the "matured" cider (a) *apiculatus* forms were absent, (b) one or two spore-forming yeasts of the Class 1 type were prominent features of the vegetation, and (c) characteristic non-sporing *Torula* forms had developed, including one of the very slow-growing class which was a feature of the corresponding "matured" ciders in the present investigation. Also, of the 15 yeast cultures isolated from the five ciders of the earlier investigation only two of the individual yeast types were duplicated, these being two forms which appeared in the 1904 Sweet Alford and the 1906 Kingston Black samples. Thus there were at least 13 different yeasts in the group of five ciders.

In the conclusions drawn from the results of this earlier work it was surmised that the larger and actively fermenting spore-forming yeasts played a prominent, if not predominant, part in the earlier stages of fermentation. The accuracy of this surmise is plainly demonstrated by the results of the present investigation. As regards the question of possible "variety" or "factory" yeast floras, the conclusions drawn from the earlier work coincide so completely with those derived from the later and more complete study that the former can be repeated here without modification to apply to both investigations.

"The results as they stand, afford no support to the ideas that certain yeasts may be regularly associated with certain apples or that the fermentations in a cider factory may be carried on mainly by any group of yeasts which may have a regular habitat there.

Regarding the former point, the Sweet Alford apple is extensively grown in Devon; so, although the samples of fruit from which the ciders in question were made came from different districts in that county, it might be expected that the organisms supposed to be common to the variety would be present on the fruit in each instance, if the supposition of their existence is justified. The likelihood of their presence would be increased by the fact that the samples did not consist of a few specimens only, but were composed in each case of about half-a-ton of the fruit, the produce, probably not of a single tree, but of many. And similarly with the Kingston Black apple. This variety is more widely grown than any other cider apple, and occurs probably in every cider-growing centre of any importance in the West of England. There are, therefore, two alternatives which arise. Either this supposed association of particular yeasts with special kinds of apples does not exist, or these common organisms have become obsolete in the ciders examined before the time of the investigation. While it would be going too far to declare absolutely in favour of the former alternative, it cannot be denied that the results point very strongly in that direction. Granting for the sake of argument the association of special yeasts with particular varieties of apples, it possesses no significance of any practical importance unless those yeasts exercise a controlling influence upon the character of the fermentation. If they are sufficiently numerous and powerful to do that, then their presence in the later stages of fermentation would be extremely likely.

But, since the results show that no common forms occur either in the three Sweet Alford or the two Kingston Black ciders respectively, the possibility of their occurrence to a significant extent at any stage of fermentation is greatly weakened; and, even admitting their presence in the earlier stages, it is difficult to see how they could impart any special character to the nature of the fermentation. Thus it appears as if the question of the occurrence of such associations is not of importance in practical cider making; and that, further, no evidence has been found to indicate their existence.

The question of the possible influence of yeasts having their habitat in the cider factory stands in some respects in the same position as the foregoing. If any single one or group of such forms played a dominant part in the fermentation, evidence of its or their existence in the bottled samples would have been expected. The only yeasts which were found in more than one sample are A and C. These were present in the 1904 Sweet Alford and the 1906 Kingston Black ciders. Since a two-years' interval elapsed between the making of these ciders, the presence of two similar yeasts in both cannot *per se* be considered to indicate that those forms dominate the fermentations at the Institute regularly; and moreover there is no evidence of their presence in the other ciders examined, either in those made in the same seasons, 1904 and 1906, or in the intermediate season of 1905.

Since the various lots of fruit came from different localities, nothing definite can be said as to whether the yeast flora of the ciders was in any way representative of the yeast flora of the district in which the fruit was grown. It may be that such was the case, in which event there would appear to be a more or less definite factor to reckon with in cider fermentation work."

In conclusion, the facts revealed by these two investigations suggest very

strongly that as a broad generalisation it is unlikely that any two ciders produced by natural fermentation have precisely the same microfloras. The individual kinds of yeasts and the relative proportions of each appear to be fortuitous and the character of the fermentation accordingly is individual to the particular cider concerned. Such a generalisation needs to be qualified in one respect, since the individual ciders under examination were made from fruit grown in different districts. It is, of course, possible that districts or individual orchards may have distinctive microfloras; but, even if that is the case, it does not follow that the precise microflora of the ciders produced from a given orchard will be identical in respect either of the individual kinds of yeasts present or of their relative dominance in the fermentation. Furthermore, since no two juices are absolutely the same in chemical composition, this variable factor may not be negligible in relation to the nutritional requirements of the respective yeasts and may accordingly influence the quantitative aspects of the mixed yeast population. So, although the locality effect might well result in the local ciders having more kinds of yeasts in common than those ciders dealt with in this paper, it would be remarkable to find any exact qualitative and quantitative duplication between individual ciders.

Nevertheless, whatever the differences in the individual microfloras may be, there is no doubt about the general similarity of the outstanding features of each phase in the fermentation life of the ciders as a whole. The prominence and persistence of relatively large, actively fermenting and spore-forming yeasts from the expression of the juice until full maturity of the end-product is common to all. The *apiculatus* phase in their earlier life is equally marked and the development of the *Zygosaccharomyces* and the *Torula* phase in later life is also definite. The resultant effect gives the product a certain character in flavour and aroma which to cider-drinkers of long standing typifies the beverage as "cider." To the scientist a fermentation so miscellaneous and uncontrolled as regards the microflora naturally appears open to improvement by the substitution of a controlled and dominant or "pure" fermentation with a selected suitable type of yeast. The advantages in uniformity and control are obvious. Such procedure has become established practice with some cider-makers, on the Continent particularly. However, to many with a long experience of English ciders such products lack, more or less, the distinctive "cider" character; they may perhaps be termed more aptly "apple wines." This fundamental difference in character is unquestionably apparent to trained palates and therefore must be accepted as a definite feature due to the nature of the difference between "dominant" and "natural" fermentation; for in some cases typical selected yeasts from British ciders have been used as the "dominant" yeasts in the trials. The question thereby raised is whether or not some cycle of a mixed microflora, analogous to that which this investigation has demonstrated, may be essential for the production of a beverage possessing the distinctive character of cider.

REFERENCES.

- (1) BARKER, B. T. P. An Investigation on the Yeast Flora of Kingston Black Ciders. Part I. *Ann. Rept. Long Ashton Res. Stn.*, 1949.
- (2) PEARCE, ELSIE B. and BARKER, B. T. P. The Yeast Flora of Bottled Ciders. *Jour. Agric. Sci.*, **3**, p. 55, 1908.

THE EFFECT OF FRUIT STORAGE ON APPLE JUICE PROCESSING.

By MARGARET E. KIESER and A. POLLARD.

It is necessary that a commercial beverage should maintain a high quality from one year to another, and also that consumers should find the beverage of their choice of fairly constant composition. Apple juice is no exception and its composition thus needs to be standardised to a considerable extent. While a certain latitude is permissible in sugar content, or to some extent in tannin content, it is undesirable that the amount of free acid should exceed fairly well defined limits. The question of acidity is of some importance, for if it rises above a certain value, the juice becomes unpalatable for the majority of consumers.

An unlimited supply of fruit of sufficiently varying types would allow this standardisation to be attained by blending the varieties, but among the apples actually available, the highly acid culinary varieties predominate. Blends incorporating a proportion of sweet low-tannin cider varieties are possible in certain years as in 1950, when there is a heavy cider crop, but a supply of such fruit cannot be counted upon in years when the crop is barely sufficient for cider production.

In some countries, such as Switzerland, the supply of fruit of a suitable composition is adequate for the demands of the juice industry and the industry has in fact been of great service in ensuring the utilisation of the fruit produced. The production of fruit specially suitable for apple juice manufacture in the United Kingdom would, however, be a long term project and its success would be dependent upon a constant demand and upon a sufficiently large scale of operation. The contribution which the industry can make, and which has constantly been emphasised in the past, is in the absorption of the better grade culls produced by the existing orchards. It is for this reason that the question of a fuller utilisation of culinary varieties such as Bramley's Seedling has again been raised.

The metabolic changes occurring in stored fruit have been the subject of extensive study by other workers and the present discussion will therefore be limited to those aspects of storage change which may be expected to affect juice processing.

The reduction of acidity which follows storage of the fruit is accompanied by a loss of water, of sugars and other soluble constituents, by pectin changes and by changes in flavour. The use of stored fruit would involve additional cost and the possible loss of fruit from rots and breakdown. The advantages to be gained from a supply of fruit of lower acidity would also have to be considered in the light of possibly increased difficulties in processing.

According to the figures published by Griffiths and Potter (1) the rate of loss of free acid from Bramley's Seedling was found greater than that of total sugar. At 3° C. the fruit lost 30% of its acidity in six months but only about 10% of its total sugar, the fruit losing at the same time about 8% of its initial weight. At 5° C. the fall in acidity was more marked being 40% in the same period while the sugar and weight losses were each about 12%. Eggenberger (2) similarly found that in fruit stored at a higher temperature the rate of loss of acid exceeded that of total sugar. A fall in acidity in Bramley's Seedling from say 1.1% to 0.8% would reduce considerably the

amount of low-acid fruit required for blending and should not involve excessive loss of sugar during the period of storage. The data of Griffiths and Potter (l.c.) also suggest that wastage of fruit need not be excessive. The more advanced state of maturity of the fruit may, however, have deleterious effects on processing operations and on juice quality and may affect the quality of the pectin subsequently extracted from the pomace (2, 3).

The work on juice concentrates carried out at Long Ashton during the war years showed that acid fruit could be used successfully for the production of low-acid concentrates by neutralisation procedures. Subsequent work, which will be described at a later date, has also shown that the de-acidification of such acid juices can be accomplished by the use of ion-exchange resins. The suggestion was made to the writers, however, that a reduction of juice acidity would more simply be obtained by longer storage of the fruit itself. At the time (in 1949) the season was already far advanced and supplies of fruit were limited; it was therefore only possible to carry out some preliminary tests. These included tests on Bramley's Seedling, on which information was specifically desired, and on some dessert varieties for the purpose of obtaining further information, not so much on acidity changes, as on the effects of storage in general on the course of juice processing.

Experimental.

Samples (20 to 40 lb.) of the four varieties—Bramley's Seedling, Crawley Beauty, Allington Pippin and Cox's Orange Pippin—were taken from a store at 3.5° C. at intervals over the period December-March for pressing. The yields of juice of the first three varieties showed little variation over this period and approached 80% of the weight of the fruit, but the Cox at the last pressing gave a very low yield due to the excessively over-ripe condition of the fruit. Samples of juice were analysed for total sugar, tannin (permanganate titration), titratable acid and pectin (as calcium pectate after acetone precipitation) and specific gravity and pH measurements were also made. Further samples of juice were treated with graded concentrations of pectin-degrading enzyme (Pectozyme) to determine the minimum amount giving juice clarification and viscosity reduction in a period of 24 hours. The main bulks of juice were treated overnight with a constant concentration of enzyme (0.1%), the same amount being used throughout to emphasise any variations in the subsequent behaviour of the juice which appeared during the fruit-storage period. The treated juices were then filtered through paper-pulp, lightly carbonated and filled into bottle under CO₂. The bottles were crown corked and pasteurised by holding for 20 minutes at 68° C. (water temperature). The samples were stored in a cool cellar in the dark.

The juice analyses are given in Table I and it will be seen that the most marked changes in composition over the storage period are those in acidity, pH and pectin content. There is a general fall in specific gravity, but the figures for total sugar in the juice show little change, losses of sugar being probably counterbalanced by water losses. The relative proportion of reducing sugar shows some increase with length of storage. There is some fall in the tannin content of the juices. The acidity in Bramley, Crawley Beauty and Allington falls, at the last testing, to about 80% of the initial value, in Cox the fall is rather greater. The figures for juice pectin show a considerable rise at the intermediate storage period and this is usually followed by a slight fall at the last testing.

TABLE I.
COMPOSITION OF APPLE JUICES AFTER DIFFERENT PERIODS OF FRUIT STORAGE.

Variety and stage of maturity	S.G.	Acidity % (as malic)	Total Sugar %	Reducing Sugar %	Pectin % (as calcium pectate)	Minimum Pectozyme concentration % required for		Tannin %	pH
						Clarification	Viscosity Reduction		
Bramley	I. 15/12/49	0.97	9.82	7.97	0.080	0.03	0.04	0.13	2.96
	II. 11/1/50	1.01	9.46	7.56	0.137	0.03	0.04	0.11	3.03
	III. 13/2/50	0.93	8.89	7.87	0.132	0.03	0.04	0.12	3.14
	IV. 29/3/50	0.79	9.23	7.92	0.112	0.03	0.04	0.12	3.13
Crawley Beauty	I. 4/1/50	0.44	10.26	8.01	0.058	0.05	0.06	0.16	3.38
	II. 13/2/50	0.42	10.48	8.67	0.139	0.07	0.12	0.09	3.51
	III. 29/3/50	0.35	10.41	9.47	0.089	0.04	0.04	0.07	3.47
Allington	I. 4/1/50	0.51	11.55	9.59	0.055	0.03	0.06	0.14	3.29
	II. 13/2/50	0.51	11.17	9.49	0.152	0.04	0.06	0.08	3.34
	III. 29/3/50	0.41	11.29	10.22	0.126	0.05	0.06	0.07	3.46
Cox	I. 4/1/50	0.50	15.47	9.85	0.060	0.03	0.08	0.12	3.52
	II. 13/2/50	0.44	15.08	11.29	0.160	0.03	0.10	0.06	3.72
	III. 29/3/50	0.35	15.58	11.66	0.179	0.05	0.06	0.05	3.53

Processing.

The amount of filtration enzyme needed to clear the juice shows no consistent relation with the amount of pectin present, but varies with the variety. The amount needed to give the maximum reduction in viscosity is usually greater than that required for clarification. In Bramley, the increasing amount of pectin present in the juice has had no apparent effect on the concentration of enzyme needed for clarification or for viscosity reduction, low concentrations sufficing for either purpose. In the other varieties the amount of enzyme needed for the reduction of viscosity is much greater than the amount which is sufficient to give clarification, but again there is no consistent relation between these amounts and the pectin content of the juice.

Storage of juice.

Appearance. After a period of 10 to 13 months in bottle the Bramley juices from the different batches of fruit showed only slight variations in appearance. Juice I pressed in December had thrown down a faint white deposit and in the two later pressings the deposit was slightly greater. The juice from the last (March) pressing had, however, no more deposit than that from the first pressing. The juice from Crawley Beauty at the first pressing was dark in colour and had thrown a definite deposit. The juices from the February and March pressings were pale in colour and were free from deposit with the exception of two individual bottles in which the juice had darkened. The Allington juices were pale in colour but all showed considerable amounts of white deposit and in the juice from the last pressing the deposit appeared during the early months of storage. In the Cox juices, which also remained pale in colour, heavy white deposits appeared in all juices after a short period of storage.

Flavour. The juices were tasted after a few months in bottle and again after the longer storage period, but as the conclusions were similar on both occasions, only the later tests will be described. The Bramley juice (I) from the first pressing was intensely acid and had little character. Juice II was similar but Juice III was slightly less acid. The juice (IV) from the last pressing was definitely less acid but otherwise similar to III. None of the juices had a marked pasteurised flavour and III and IV were preferred to I and II.

The Crawley juices had more character at the last testing than after a short period of storage. Juice I was sweet but not excessively so, Juice II was very similar but III was insipid and lacking in fruit character. The Allington juice I was very aromatic but had a slight pasteurised flavour, II was very similar but rather thinner, while III was lacking in acidity and had a rather abnormal flavour. The Cox juice I from the first pressing had a flavour which was not typical of the variety, Juice II was better but was very sweet, while Juice III was excessively sweet and syrupy. None of the juices had the characteristic flavour of long-stored fruit.

Discussion.

The most marked effect of fruit storage has thus been to lower the acidity of the juice. In Bramley the effect has been beneficial rather than otherwise and in these tests storage of the fruit has not been followed by additional processing difficulties or by appreciable increased deposition in bottle. In Crawley Beauty the fall in acidity has been accompanied by some loss of juice quality and in the varieties Allington and Cox over-maturity of

the fruit has been accompanied by the increased formation of storage deposits in the juice.

The formation of deposits in bottle on storage has been a frequent source of trouble in apple juice preparation and particularly when there has been no intermediate period of storage of the juice in bulk. The deposits appear to be of two main types: a dark coherent deposit associated with oxidative juice changes as in the Crawley juice I, and a pale pectinous deposit of the type found in the Cox juices. The former type of deposit has in our own tests often been found in juices bottled in the presence of air. To test this point further a juice prepared from a mixture of culinary and dessert fruit was divided into batches, one series being bottled with an air space and the other carbonated and bottled under CO_2 . After pasteurisation and storage for 15 months the former juice showed extensive browning and the formation of a dark deposit while the carbonated batch remained pale in colour and showed merely a very faint deposit visible on shaking.

In the juices prepared from stored fruit in the present tests the deposits (in Allington and Cox) have been of the second type and have had the appearance of a degraded pectin, an assumption which has been confirmed by the analyses of some such deposits. A sample of the deposit from the Cox Juice II was collected by means of the centrifuge and washed with successive portions of 70% acetone. The dried material gave a calcium pectate figure corresponding to about 0.2 g. of deposit per litre of juice and had a methoxyl content of 2.4%, which corresponds to a degraded pectin product.

From the point of view of stability, the amount of pectin left in the juice appears of less importance than its degree of degradation; the addition of pectin has indeed been recommended as a stabilising agent (4). The relation between the state of degradation of the pectin and juice stability has been further brought out by analyses of some other juices and deposits. A batch of juice prepared from a mixture of culinary and dessert fruit in January (1950) and which had been treated with filtration enzyme, after about eight months' storage, threw down a deposit similar in appearance to that found in the Cox juice. It gave, in fact, a similar analysis but more nearly approached the composition of pectic acid (101.4% calcium pectate, 1.4% OCH_3). In contrast, a juice prepared from mixed fruit earlier in the season (October, 1949) by filtration alone and without the addition of enzyme remained clear over a period of 15 months, still contained its original pectin (0.165%) and showed no change in viscosity. The pectin isolated from this juice at the end of the storage period still appeared undegraded, having a methoxyl content of 10.3% of the calcium pectate figure.

The effect of a partial pectin degradation on juice stability is shown by the behaviour of another batch of the juice prepared from the Cox fruit at the second storage period. This juice had shown considerable self-clarification without the addition of enzyme and it was accordingly allowed to clear spontaneously and was then filtered through pulp before bottling with CO_2 . On storage the juice remained clear for some months and then formed an extensive gel. Analysis of this gel showed that it was mainly pectin (calcium pectate 78%) which had been partially demethylated, having a methoxyl value of 5.3% of the calcium pectate figure. It had thus been sufficiently degraded to form a pectic acid type of gel under favourable conditions.

A study of the pectase content of apple varieties (5, 6) suggests that the behaviour of the juices from stored fruit is related, not only to their increased pectin content, but also to their pectin enzyme content. The varieties

Bramley's Seedling and Crawley Beauty which did not throw appreciable deposits on storage of the juice were found virtually lacking in pectase. The dessert varieties in general, and including Cox and Allington, have been found rich in the enzyme. The presence of fruit pectase should normally assist the juice clarification produced by the addition of filtration enzymes (7), but the present tests show that if the juice is rich in pectin and in pectase and if the amount of added enzyme is small, then the course of juice clarification may be deceptive. The pectin degradation which takes place may be sufficient to give clarification and a reduction in viscosity, but may leave in solution a partially degraded pectin material which eventually precipitates on storage of the juice. The need for adequate control of the clarification process has been emphasised by Reid (8).

The pectin changes occurring during the clarification of the Cox juices closely resemble a natural clarification or defecation (5, 9) and the main effect is pectin demethylation, which is accompanied by only a partial break up of the pectin molecule due to the action of the polygalacturonase in the added filtration enzyme. Other tests on juices of this type have shown that unless fairly high concentrations of filtration enzyme are added, the pectin is liable to be thrown out of solution (as a pectic acid) before it can be sufficiently degraded by the added enzyme.

The marked stability to heat shown by the pectase normally present in some apple juices (6 and unpublished work) would also suggest the possibility of further pectin changes taking place in bottle after pasteurisation and leading to increased deposit formation.

Conclusions.

The tests have thus shown that the acidity of apple juice can be lowered by deferring pressing until the fruit is in a more advanced state of maturity. In some dessert varieties the changes in juice composition give rise to increased difficulties in processing and juice storage, but in the high-acid variety Bramley's Seedling such difficulties have not been apparent. Further tests on other culinary varieties are being undertaken to determine how far acidity reduction can be allowed to proceed before processing difficulties are to be expected to occur.

Acknowledgment.

We wish to express our thanks to Miss M. E. Moseley for assistance in the experimental work.

REFERENCES.

- (1) GRIFFITHS, D. G., POTTER, N. A. and HULME, A. C. *J. Hort. Sci.* 1950, **25**, 266.
- (2) EGGENBERGER, W. *Ber. der Schweiz. Bot. Ges.* 1949, **59**, 91.
- (3) BURROUGHS, L. F., KIESER, M. E., POLLARD, A. and STEEDMAN, J. *Ann. Rept. Long Ashton Res. Sta.*, 1943, 136.
- (4) MOTTERN, H. H., NEUBERT, A. M. and EDDY, C. W. *Fruit Prod. J.*, 1940, **20**, 36.
- (5) KIESER, M. E., POLLARD, A. and STONE, A. M. *Ann. Rept. Long Ashton Res. Sta.*, 1948, 228.
- (6) POLLARD, A. and KIESER, M. E. *J. Sci. Food and Agr.*, 1951, **2**, 30.
- (7) JANSEN, E. F. and MACDONNELL, L. R. *Arch. Biochem.*, 1945, **8**, 97.
- (8) REID, W. W. (1950). Recent Advances in Fruit Juice Production, by V. L. S. Charley and others. *Commonwealth Bureau of Horticulture and Plantation Crops, Tech. Comm. No. 21*, 145-161.
- (9) BEECH, F. W. and CHALLINOR, S. W. *This journal* p. 143.

THE EFFECT OF MANURIAL TREATMENT ON THE COMPOSITION OF BLACKCURRANTS.

By MARGARET E. KIESER, A. POLLARD and C. F. TIMBERLAKE.

Trials have been carried out over a number of years to study the effect of manurial treatment on the composition of blackcurrants. In the two varieties Cotswold Cross and Mendip Cross, there has been a general tendency for increasing nitrogen uptake by the bush to be associated with a lower ascorbic acid content in the fruit. This effect has, however, been counter-balanced by an increase in the quantity of fruit produced with increase of nitrogen, and by an improvement in its general quality (1, 2).

The setting up of a manurial trial to study the effect of both N and K manuring on the variety Baldwin has enabled these tests of fruit quality to be extended to this variety. These bushes, already established for several years, had previously received a complete manurial treatment including dressings of farmyard manure. The subsequent N and K treatments would therefore not be expected to produce any immediate marked differences in the nutritional status of the bushes. Exploratory tests on fruit quality have, however, been made and the results compared with such differences in nutrient status as have appeared in the leaf analyses.

Experimental.

The manurial treatments consist of five levels of N, viz. 2, 4, 6, 8 and 10 cwts. ammonium sulphate per acre, applied at two levels of K, viz. 1 and 3 cwts. potassium sulphate per acre, comprising ten treatments in all and each being replicated five times. The tests therefore involved sampling 50 small plots from which fruit was to be drawn for analysis. The earlier tests of this type had emphasised the difficulties arising in sampling fruit from numerous plots simultaneously, more especially when the analyses involve relatively unstable constituents such as ascorbic acid. It is desirable to obtain samples in fresh condition without at the same time spreading the picking period and introducing differences in the ripeness and condition of the fruit.

A preliminary test on an earlier variety showed that weighed samples of carefully strigged berries sealed immediately in cellophane bags and rapidly frozen in a deep-freeze cabinet at -10° F. showed little change in composition over a period of a week. The values for ascorbic acid (as mg. per 100 g.) found for samples taken from the fruit of one bush and thus stored were as follows: Original value, 194; after two days, 195; after four days, 197; after six days, 196. These differences were no more than would be expected as a result of sampling and experimental errors and this method of fruit storage was therefore adopted for the tests on Baldwin.

All fruit was picked on one day and from each separate batch of fruit 50 g. samples were taken for the determination of ascorbic acid. Larger bulks were taken for additional analyses as described later. All samples were rapidly frozen and stored.

Results.

Ascorbic acid. The ascorbic acid determinations were carried out as described in the earlier papers and were completed within four days. The results are summarised in Table I.

TABLE I.
ASCORBIC ACID (MG./100G.)—MEANS OF 5 REPLICATE PLOTS.

Rate of K application (cwts./acre)	Rate of N application (cwts./acre)					Mean
	2	4	6	8	10	
1	230.4	191.0	205.6	206.8	194.6	205.7
3	230.4	216.2	207.6	189.6	200.8	208.9
Mean	230.4	203.6	206.6	198.2	197.7	

		P < .05	P < .01	
Significant	{	means of 5	17.91	23.82
Difference		means of 10	12.66	18.84

The analysis of these results led to the following conclusions:—

(a) No significant difference was to be attributed to variation between replicates, i.e. to position in the field.

(b) The lowest rate of N application gave a higher level of ascorbic acid in the fruit than all other rates of application. Fruit samples from other treatments (4 to 10 cwts./acre) did not show significant differences.

(c) At the N_4 level the higher rate of K application gave a significantly higher ascorbic acid content. At the other N levels no differences due to K were apparent.

Other constituents. Some exploratory analyses were carried out on other fruit constituents, viz., sugar, pectin, titratable acid and total nitrogen, together with pH determinations. Earlier tests had shown no consistent effects on these resulting from manurial treatment, although in some years there was a tendency for higher sugar content to be associated with a low nitrogen status of the bush. In the present tests these estimations were only made on bulked samples from each manurial treatment (not on each replicate), but with the exception that in one treatment the five replicates were also analysed separately. The means of the analyses of these last replicates agreed well with the analysis of the bulk sample but showed that there was considerable variation between individual replicate analyses.

These analyses will not be given in detail here, but it may be stated that the maximum variations found in sugar, pectin and nitrogen were about 7% from the mean values, and the values for pH and acidity showed little variation between treatments.

Discussion.

As the N and K treatments have not yet been applied over a long period, major differences in fruit composition would not be expected, nor would the nutrient status of the bushes be expected to follow the treatments closely. It is of interest, however, to compare the fruit analyses so far obtained with the actual nitrogen status of the bushes in the same season (1950). In the first line of Table II the numbers of the manurial plots are listed in order of increasing nitrogen status (from figures for leaf nitrogen supplied by Dr. C. Bould), the two levels of potash being taken separately. The plots are also listed below in order of increasing or decreasing concentrations of fruit constituents as indicated.

TABLE II.
RELATIONS BETWEEN NITROGEN STATUS OF BUSH AND FRUIT COMPOSITION.

	Plots at K ₁ level	Plots at K ₂ level
Leaf N increasing	1, 4, 5, 3, 2	7, 6, 8, 9, 10
Fruit N increasing	1, 4, 3, 2, 5	8, 6, 7, 10, 9
Ascorbic acid decreasing	1, 4, 3, 5, 2	6, 7, 8, 10, 9
Sugar decreasing	1, 5, 4, 3, 2	6, 10, 9, 7, 8
Pectin decreasing	1, 3, 5, 4, 2	6, 10, 7, 9, 8

It will be seen that there is a tendency for the level of fruit N to correspond to that in the leaf. At the lower K level the lowest leaf N is in Plot 1, which received the lowest application of N, and this shows the lowest value for fruit N. Fruit from Plot 1 also has the highest concentration of ascorbic acid (as brought out more fully in Table I) and also the highest concentrations of sugar and pectin. Similarly fruit from Plot 2 with the highest leaf N has the lowest ascorbic acid, sugar and pectin. At the higher K level the results are inconclusive.

There is as yet no definite relation apparent between K manuring and fruit composition. As shown in Table I the higher rate of application of K gave a higher ascorbic acid content at one N level, but this was not found at the other N levels or for the manurial plots as a whole. A comparison of these fruit analyses with the figures for leaf K in the previous year (1949) showed no consistent relation between K status and fruit composition except that the fruit with the lowest ascorbic acid came from plots with the lowest K status.

The conclusions to be drawn from the present tests are therefore that there is again a general tendency for low N status of the bushes to be associated with higher ascorbic acid and vice versa, some of the differences being significant; and that the figures for sugar and pectin follow those for ascorbic acid. The effect of K manuring on fruit composition is as yet not clearly established, but it is possible that additional K may favour an increase in ascorbic acid.

Acknowledgments.

We wish to express our thanks to Miss H. Ferres for assistance in the statistical treatment of the results and to Miss M. R. Moseley for assistance in the experimental work.

REFERENCES.

- (1) BRYAN, JOAN D. and POLLARD, A. *Ann. Rept. Long Ashton Res. Sta.*, 1947, 216-221.
- (2) KIESER, MARGARET E., POLLARD, A. and STONE, AUDREY M. *ibid.*, 1949, 159-162.

A COMPARISON OF SOME VARIETIES OF FRUIT PRESERVED BY BOTTLING, CANNING AND FREEZING.

PROGRESS REPORT I.

By ALICE CRANG, LORNA KENDALL and MARGARET STURDY.

A comparison has been made of the preserving qualities of 24 different varieties of soft fruit when preserved by bottling, canning and deep freezing during 1950. In addition, the results of bottling and canning trials on plums preserved in 1949 are given.

Experimental.

Fruit Used.

Unless otherwise stated, all the fruit used had been grown at Long Ashton Research Station. Quickly perishable fruits were processed on the day of picking, and other fruits were either processed the same day, or left overnight in a cool store. The pears were kept in store until considered to be sufficiently ripe for canning.

Bottling Methods.

In all the tests, 1 lb. all-glass screw-band type of preserving jars were used. The jars of fruit were processed by immersing in cold water which was heated to either 165°, 180° or 190° F., and held at these temperatures for the time required for each fruit.* The jars were stored in semi-darkness at room temperature.

Canning Methods.

Fruit lacquered cans were used for all fruits unless otherwise stated. A2½ sized cans were used in the 1949 preserving season, but 1 lb. cans during 1950, so that the weights of fruit and syrup used in the latter could be the same as in the bottles*. The cans were stored alongside the bottles.

Freezing Methods.

An ice cream storage cabinet was used for freezing and storing the fruit. The fruit was frozen at -10° F. and stored at temperatures ranging from -10° to 0° F.

The freshly prepared fruit was put into bags made from "Cellophane," grade MSAT300/30, the required amount of sugar or cold sugar syrup added, and the bag heat-sealed after removing as much air as possible. The bags of fruit were put into waxed cartons for immediate freezing. The cartons were kept below 0° F. until thawed for tasting.

Sugar or Syrup.

Unless otherwise stated, 40° Brix cane sugar syrup was used. The proportion of fruit to syrup by weight in the bottled and canned fruit was 1.27 : 1. The ratios used for the frozen fruit were 1.6 : 1 of fruit to sugar syrup, and 4 : 1 of fruit to sugar, giving the same proportion of sugar and fruit in each of the two freezing processes.

Judging Methods.

The fruit was judged after three to six months' storage, and some again after about a year's storage.

* See Ministry of Agriculture Bulletin 21, 7th Edition, for processing details.

Frozen fruit of kinds which are usually eaten raw, was allowed to thaw for four hours at room temperature before judging against the same varieties from freshly opened bottles and cans. The blackcurrants and gooseberries were cooked gently until tender in the syrup in which they were frozen, then cooled before tasting. The frozen blackberries were judged both raw and cooked.

No individual marks are given for the fruit judged in 1949, as a numerical scoring system was not used, and so comparisons are difficult. The outstanding points of quality have however, been included in the comments under each variety.

During the 1950 season the same six people judged each of the fruits, which were put in identical glass containers and marked by a code number for judging. Usually three varieties, processed by three methods, were judged at a time. If more than three varieties of one kind were preserved, the bottled fruits of each variety were judged together a second time, to allow for any variation in tasting from day to day.

The following system of marking was used during the 1950 season :—

Colour.	10	Excellent.	
	9	Unusually even and bright.	
	8	Average.	
	7	Some unevenness or slightly too pale or too dark.	
	5-6	Unattractive and patchy, not objectionable.	
	0-4	„ „ „ objectionable.	
Texture.	10	Excellent.	
	9	Unusually tender and whole.	
	8	Average.	
	7	Slight unevenness, softness, breakdown or slightly tough.	
	5-6	Uneven, too hard or too soft, but passable.	
	0-4	Too hard or too soft, not passable.	
Flavour.	10	Unusually full rich flavour.	
	9	Very good.	
	8	Normally good.	
	7	Rather weak.	
	5	Very weak or slightly foreign.	
	0-4	Poor or foreign flavour.	
Sugar/Acid.	Either section A or section B marked.		
	A5	Sufficiently sweet, not too sour.	B5 as A5.
	A4	Slightly over sweet.	B4 Slightly sour.
	A3	Moderately over sweet.	B3 Moderately sour.
	A2	Too sweet.	B2 Sour.
	A1	Very sweet.	B1 Very sour.
Size.	5	Very good for preserving, even.	
	4	Good, fairly even.	
	3	Average, or rather too large or too small.	
	2	Under average, uneven or too large or too small.	
	1	Poor, or much too large or too small.	
Total.	Maximum 40.		

Results.

Blackberries.

The varieties Ashton Cross, Himalaya Giant and Merton Early were preserved by bottling, canning and freezing in 1950. Two series were judged, after three and five months' storage respectively. The average of 36 scores for the three varieties, and 18 for each process are given in Table I.

TABLE I.
BLACKBERRIES.

Variety. Average of all Processes	Storage (months)	Colour (10)	Texture (10)	Flavour (10)	Sugar/ Acid (5)	Size (5)	Total (40)
Ashton Cross		7.5	7.6	7.5	4.2	3.8	30.6
Himalaya Giant		8.1	7.6	7.4	4.2	4.3	31.6
Merton Early		7.3	7.5	7.2	4.2	4.4	30.6
Process. Average of all Varieties							
Bottled	3	7.3	7.6	7.7	4.5	4.4	31.5
„	5	6.5	7.3	7.0	3.8	4.1	28.8
Canned	3	7.8	7.8	8.3	4.6	4.2	32.7
Frozen with sugar uncooked	5	7.8	7.8	7.0	4.6	4.2	31.4
„ with syrup uncooked	3	8.1	7.1	6.1	3.5	4.6	29.4
„ with syrup cooked	5	8.2	7.7	8.0	4.4	3.7	32.0

Varieties.

Ashton Cross. The fruit from this variety was rather smaller than the other two, but there was little difference in other qualities.

Himalaya Giant. The scoring for this fruit was found to be significantly higher than either of the others, chiefly due to its better colour when preserved.

Merton Early. Slightly poorer in colour and flavour than the other varieties.

Preserving Methods.

The bottled fruit deteriorated, particularly in colour and flavour, during the longer storage. The canned fruit was similar to the bottled fruit after three months' storage.

The fruit frozen in syrup and cooked before tasting was preferred to the uncooked fruit. The uncooked fruit frozen in sugar was better, even after two months' longer storage, than the uncooked fruit in syrup.

Freezing was definitely superior to heat-processing for retaining the colour of the fruit.

Blackcurrants.

Ten varieties of this fruit were compared after preservation by bottling and canning during the 1949 season, and seven varieties during 1950. In the latter season, they were also preserved by freezing. The proportion of fruit to syrup used in each process was 1.27 : 1.

As there was no significant difference between the total marks given when comparing bottled and canned blackcurrants, though in general the bottled fruit was slightly better in colour and the canned fruit was of better texture, the results have been combined for the average figures for heat-processed fruit given in Table II. The frozen blackcurrants were cooked gently in the syrup in which they were preserved, then cooled to room temperature before being judged.

TABLE II.
BLACKCURRANTS.

Variety	Colour (10)		Texture (10)		Flavour (10)		Sugar/Acid (5)		Size (5)		Total (40)	
	HP*	F†	HP	F	HP	F	HP	F	HP	F	HP	F
Baldwin ..	7.9	6.2	8.3	6.6	8.2	7.2	B4.5	B4.2	4.4	2.8	33.3	27.0
Cotswold Cross ..	8.6	7.8	8.5	8.2	7.9	6.4	B4.7	5.0	4.6	3.0	34.3	30.4
Malvern Cross ..	7.3	7.4	8.3	8.8	7.2	7.0	A4.9	B4.4	4.5	5.0	32.2	32.6
Mendip Cross ..	7.3	6.7	8.1	7.5	7.5	7.5	B4.4	B4.7	3.6	3.0	30.9	29.4
Seabrook ..	7.8	6.8	7.9	8.0	8.1	8.0	B3.7	B4.0	4.3	3.3	31.8	30.1
Westwick Choice	8.1	7.5	8.6	7.0	7.6	7.0	B4.2	B3.8	4.5	3.8	32.9	29.1
PS/24/1922+ ..	8.1	7.7	8.9	6.8	8.3	6.7	B4.9	B3.8	4.3	4.3	34.6	29.3
Average (all varieties) ..	7.9	7.1	8.4	7.6	7.8	7.1	B4.5	B4.3	4.3	3.6	32.9	29.7

*HP Heat-processed (bottled and canned), average of 18 scores.

†F Frozen, average of 6 scores.

+ Long Ashton Seedling.

From these figures it will be seen that the heat-processed fruit gained higher average marks in each section than the frozen fruit. The differences between the total figures for these processes were significant.

Varieties.

Baldwin. A good blackcurrant, above the average in the bottling and canning trials in both seasons, but lowest in the freezing trials.

Boskoop Giant. (1949 trials only). Lighter in colour than most and rather too soft in texture for heat-processing. Average flavour.

Cotswold Cross. This variety was above the average in all methods of preserving tested. It was considered the best for colour in both seasons. Its flavour, said to be reminiscent of blackcurrant leaves, was liked by some judges, but not by others.

Davidson's Eight. (1949 trials only). Fairly good in colour and texture. Fair flavour, which was retained quite well after opening.

Edina. (1949 trials only). This variety was judged one of the best when the container was first opened, but its flavour deteriorated after two or three hours. Rich dark red colour, good soft texture, and sweeter fruit than most.

Malvern Cross. Quite a good variety in both season's trials but not of outstanding quality. Fruit sweeter than most. Retained its shape and texture well when frozen.

Mendip Cross. This variety did not score as highly as most in either season's trials, but in the 1950 season it was the first variety picked and the fruit was in poorer condition than the later varieties.

Seabrook. A very good flavoured blackcurrant but noticeably more acid than most in both season's trials. A good early variety.

Westwick Choice. This blackcurrant was judged to be the poorest in flavour in the bottling and canning trials in 1949, and of average quality in 1950.

Westwick Triumph. (1949 trials only). The fruit was a good colour, and fairly good flavour, which however deteriorated after the cans or bottles had been opened a short time.

Long Ashton Seedling PS/24/1922. (1950 trials only). This variety scored the greatest number of marks in bottling and canning trials. It is one of the late season blackcurrants.

Gooseberries.

Three varieties of gooseberries, Keepsake, Leveller and Whinham's Industry, have been compared for preserving quality in two seasons. For this purpose the fruit was picked while the berries were hard and green.

Sugar Syrup (30° Brix) was used in the usual proportions in each method of preserving. Fruit lacquered cans were used in 1949 and plain cans in 1950.

Table III gives the average results of 18 scores for the three varieties and for the three methods of processing used in 1950.

TABLE III.
GOOSEBERRIES.

Variety. Average of all Processes	Colour (10)	Texture (10)	Flavour (10)	Sugar/Acid (5)	Size (5)	Total (40)
Keepsake	7.3	7.4	7.3	3.5	3.8	29.3
Leveller	7.5	6.4	7.0	4.3	4.0	29.2
Whinham's Industry	6.0	6.6	7.1	4.0	2.5	26.2
Process. Average of all Varieties						
Bottled fruit ..	7.5	6.0	6.8	4.3	3.5	28.1
Canned fruit ..	5.8	7.5	7.6	4.5	3.4	28.8
Frozen fruit ..	7.6	6.8	6.9	B3.1	3.4	27.8

Varieties.

Keepsake. This variety proved a good one for preserving in both season's trials. The colour is a light green.

Leveller. The fruit when picked before it has fully developed, has proved as satisfactory as Keepsake for preserving. It might be useful to preserve the "thinnings" of this variety and leave the remaining fruit to ripen for dessert use.

Whinham's Industry. This variety was inferior to the previous ones in both season's trials. Its colour was dull green.

Preserving Methods.

Although the total scores given in the three methods of processing are very similar, the allocation is different. The colour of the canned fruit was poor even when plain cans were used, and fruit in lacquered cans in 1949 was awarded only 4.5 marks compared with 7.7 for bottled fruit stored for the same length of time. On the other hand the texture and flavour of the canned fruit were superior to that of either the bottled or frozen fruit.

The proportion of sugar used for the frozen fruit was not sufficient, but the higher proportion of 30° Brix syrup used in the heat-processed gooseberries seemed satisfactory.

Loganberries.

The loganberries were processed by four different methods: bottling, canning, freezing with sugar syrup and freezing with sugar. The average marks of the six judges are given in Table IV.

TABLE IV.
LOGANBERRIES.

Process.	Colour (10)	Texture (10)	Flavour (10)	Sugar/Acid (5)	Size (5)	Total (40)
Bottled	7.5	7.7	9.0	B4.5	4.8	33.5
Canned	6.7	7.5	8.7	B4.8	4.8	32.5
Frozen with Syrup ..	8.0	8.3	7.3	B3.2	5	31.8
Frozen with Sugar ..	7.3	8.3	7.0	B2.9	5	30.5

It will be seen that the heat-processed fruit was given preference, but the differences between the total marks were not significant. A higher proportion of sugar is needed for the frozen products to counter-balance the acidity of the fruit.

The quality of the frozen packs deteriorated appreciably during the hour in which they were being judged.

Pears.

As is well known, this fruit needs careful preparation if oxidative changes are to be prevented. They may be retarded to some extent by holding the fruit in a dilute solution of citric acid or brine prior to heat-processing, but the addition of ascorbic acid to the syrup covering the fruit has been recommended when freezing this fruit.

Preliminary trials were made using Doyenné du Comice pears. The fruit was put either in 2% citric acid solution or 2% brine, as soon as prepared, and left there while the remaining fruit was peeled. Ascorbic acid was added to the sugar syrup for some of the freezing tests in the proportion of 150 mg. per 1 lb. of total contents. The ratio of fruit to 40° Brix syrup for the quarters and slices was 1 : 1 and 2 : 1 respectively, and plain cans were used for the canned fruit. Since there was little variation in the sugar acid and size markings (average A4.0 and 4.0 respectively), these have been omitted from Table V. The results are the average marks of six judges.

TABLE V.
PEARS—PROCESSES.

Process	Storage (months)	Citric acid rinse	Ascorbic acid in syrup	Colour (10)	Texture (10)	Flavour (10)	Total (40)
Slices Frozen	3	+	—	7.0	7.0	6.7	28.7
" "	3	+	+	8.7	7.0	7.3	31.0
" "	14	—	—	3.0	5.3	5.2	21.0
" "	14	+	—	4.8	6.2	6.0	25.3
" "	14	—	+	7.7	5.5	5.8	26.2
" "	14	+	+	8.0	5.5	5.7	27.2
" Canned	14	—	—	4.0	7.7	6.3	26.0
" "	14	+	—	7.7	8.2	7.3	31.7
Quarters Frozen	14	—	—	5.3	5.0	5.3	24.0
" "	14	+	—	6.2	5.8	5.3	25.3
" "	14	—	+	7.2	5.5	5.2	26.7
" Canned	14	—	—	5.8	8.2	7.5	29.8
" "	14	+	—	6.8	8.3	6.5	30.2

From Table V it will be seen that ascorbic acid makes a greatly improved frozen product, and a citric acid rinse was beneficial for the frozen and canned fruit.

Three other varieties of pears were preserved in quarters for comparison of the frozen and bottled fruit. The frozen fruit was kept in 2% citric acid solution during preparation and ascorbic acid was added to the syrup (150 mg. per lb. of contents). The fruit for bottling was put in 2% salt during preparation, and no acid was added to the syrup. The fruit was judged after three months' storage. The average of six scores are given.

TABLE VI.
PEARS—VARIETIES AND PROCESSES.

Variety	Process	Colour (10)	Texture (10)	Flavour (10)	Sugar/ Acid (5)	Size (5)	Total (40)
Bristol Cross	Bottled	7.5	8.3	6.8	A4.5	4.3	31.5
" "	Frozen	6.3	5.5	4.3	A4.5	3.7	24.3
Conference	Bottled	6.7	8.3	8.0	A3.5	3.8	30.3
"	Frozen	6.5	7.2	5.3	A4.0	3.7	26.7
Pitmaston Duchess	Bottled	5.3	7.8	7.3	A4.2	3.2	27.8
" "	Frozen	7.8	6.8	3.3	A4.2	3.3	25.4

It will be seen that the texture and flavour of the frozen fruit was decidedly inferior to the same variety preserved by bottling. The variety Pitmaston Duchess was the least satisfactory of the bottled fruit in these trials.

Plums.

The varieties Giant Prune and Warwickshire Drooper were preserved in 1950, and the other varieties in 1949. Freezing trials were not included. The fruit preserved in 1950 was judged after three months, and that preserved in 1949 was also judged after 15 months' storage. The average of 12 scores for variety and 72 for method of processing are given in Table VII.

TABLE VII.
PLUMS.

Variety. Average of both Processes	Colour (10)	Texture (10)	Flavour (10)	Sugar/ Acid (5)	Size (5)	Total (40)
Apricot Gage	8.3	8.0	7.4	B4.5	4.5	32.7
Belle de Louvain	5.6	6.9	6.0	B4.5	3.0	26.0
Blaision Red	6.8	8.6	6.8	B4.1	3.6	29.9
Frome Cross	7.6	7.2	7.6	B4.7	4.5	31.6
Giant Prune	6.7	5.5	6.8	A3.8	2.4	25.2
Pershire (Yellow Egg) ..	8.1	8.1	6.5	B4.8	4.4	31.9
Purple Pershire	6.4	7.9	6.8	B4.1	3.8	29.0
Severn Cross	7.2	6.8	6.1	A4.0	2.0	26.1
Shropshire Prune Damson	8.0	7.6	8.3	B4.0	4.1	32.0
Thames Cross	8.0	6.6	6.9	A4.1	2.2	27.8
Victoria	8.0	8.5	7.3	B4.9	3.8	32.5
Warwickshire Drooper ..	7.4	8.3	7.8	B4.6	3.7	31.8
Process. Average of all Varieties						
Bottled Fruit	7.2	7.1	6.8	4.3	3.6	29.0
Canned Fruit	7.5	7.9	7.2	4.4	3.4	30.4

The canned fruit was slightly better in quality than the bottled fruit from all aspects except size.

Varieties.

Apricot Gage. This variety attained the highest total score. It has an apricot coloured flesh and slight apricot flavour. (This fruit was grown at Evesham).

Belle de Louvain. One of the least attractive of the series when bottled or canned.

Blaision Red. This variety proved of fair quality only for bottling and canning. (Obtained locally but not grown at the Research Station).

Frome Cross. A new variety which has proved consistently good in bottling and canning trials. It has a damson colour and flavour which were well retained in the preserved fruit.

Giant Prune. Not a good plum for preserving. It is too large and lacked characteristic flavour after processing.

Pershore (Yellow Egg). This fruit was good in appearance, but the strong kernel flavour especially noticeable in the bottled fruit was not liked by all.

Purple Pershore. The fruit was of fair quality when preserved, but below the average in colour.

Severn Cross. This variety is essentially a dessert fruit. It is too large for canning whole, and its delicate flavour is lost in preserving.

Shropshire Prune Damson. This damson compared very favourably with the varieties of plums tested.

Thames Cross. Like Severn Cross, this fruit is large, sweet and lacking in acid when preserved. It is essentially a late dessert plum.

Victoria. Once again this variety has proved one of the best for canning and bottling, though the flavour of the bottled fruit was disappointing.

Warwickshire Drooper. Another very good plum for preserving.

Raspberries.

Norfolk Giant, Mallings Promise and Mallings W/51/57 were the three raspberry varieties processed by bottling, canning and freezing with sugar or with syrup in 1950.

The average of 36 scores for the varieties and 18 for the heat-processed and frozen fruit are given in Table VIII, since there was no significant difference within these groups.

TABLE VIII.
RASPBERRIES.

Variety. Average of both Processes	Colour (10)	Texture (10)	Flavour (10)	Sugar/ Acid (5)	Size (5)	Total (40)
Mallings Promise	7.7	8.2	7.2	4.6	4.0	31.7
Mallings W/51/57	8.1	8.1	7.4	4.5	3.8	31.9
Norfolk Giant	7.6	8.1	7.8	B4.2	4.2	31.9
Process. Average of all Varieties						
Heat-processed	6.8	8.6	7.7	4.5	4.0	31.6
Frozen fruit	8.7	7.6	7.3	4.3	4.0	31.9

As will be seen, there was little difference between the different varieties tested, though Norfolk Giant gained highest marks for flavour, in spite of being considered more acid than the other two.

The frozen fruit was greatly superior in colour, but poorer in texture and flavour than the heat-processed fruit. The fruit had been picked after rain, and although carefully selected before preserving, a mouldy flavour was detected in some of the frozen fruit. A higher proportion of sugar to fruit used in freezing would probably increase the superiority of the frozen over the heat-processed fruit.

Strawberries.

Two varieties of strawberries (Royal Sovereign and Auchincruive Climax) were preserved by bottling and canning in 1949, and a third variety, Huxley, was included in the 1950 trials. The Auchincruive Climax fruit was obtained from Ledbury and the Huxley from Brockley. The 1950 trials included freezing the fruit with sugar and sugar syrup.

The syrup used in the bottled and canned fruit was coloured with Ponceau 2R in 1950; this fruit was judged against the undyed frozen fruit after four months' storage.

Since the results obtained in the heat-processed and frozen fruit were so different with the different varieties, the average scores for each of these processes is given in Table IX.

TABLE IX.
STRAWBERRIES.

Variety and Process	Colour (10)	Texture (10)	Flavour (10)	Sugar/Acid (5)	Size (5)	Total (40)
Auchincruive Climax Heat-processed ..	7.5	8.0	6.8	A4.3	3.7	30.3
Auchincruive Climax Frozen	9.3	8.0	8.2	B4.2	4.1	33.8
Huxley Heat-processed ..	7.7	7.0	5.9	A2.8	3.4	26.8
Huxley Frozen	8.4	6.8	4.2	B4.2	4.0	27.6
Royal Sovereign Heat-processed ..	7.8	7.9	7.7	A4.4	3.7	31.4
Royal Sovereign Frozen	7.5	8.2	7.5	B4.0	3.3	30.5

Varieties.

Auchincruive Climax. This proved to be the best of the three varieties of strawberries tested for freezing. Its appearance and flavour did not deteriorate greatly after it had been thawed for three hours. In the 1949 trials the bottled and canned fruit was judged slightly superior to Royal Sovereign, but this order was reversed in 1950.

Huxley. The poor flavour of this strawberry was very noticeable in each method of preserving used. The total marks awarded were significantly less than those for the other varieties.

Royal Sovereign. This variety proved better for bottling or canning than for freezing, as the fruit collapsed and lost its attractive colour very quickly after thawing. The flavour was superior to the other varieties in the bottled or canned fruit.

Processing.

The total marks awarded to the strawberries frozen with syrup or sugar (31.0 and 30.4 respectively) were significantly higher than that for the canned or bottled fruit (29.7 and 29.3 respectively). The additional marks of the frozen fruit were chiefly gained in its superior colour, as although the dyed

bottled or canned fruit was liked in preference to the undyed fruit, its colour was not very natural.

Discussion.

The preserving quality of the different varieties of fruit varied considerably from one kind to another. The differences were small in the blackberries, blackcurrants and raspberries tested, but were definite in the gooseberries, pears, plums and strawberries. Newer varieties which were worthy of notice were Apricot Gage and Frome Cross plums, both of which were consistently good in bottling and canning trials in two seasons, and the Auchincruive Climax strawberry which proved excellent for freezing and quite good for bottling and canning, confirming other preserving tests on this variety (1). The two newer dessert varieties of plums, Severn Cross and Thames Cross both gave poor quality products when preserved. Other tests have also shown the unsuitability of these two varieties for preserving (2).

The method of freezing and storing on a small scale in one cabinet for the whole process, with a temperature range of -10° F. to 0° F., proved quite satisfactory. The most noticeable advantage of freezing over bottling and canning was in retaining the natural colour of the fruit, but in several kinds of the frozen fruit the texture or flavour was not liked so well. A higher proportion of sugar to fruit seems to be required than the 1 : 4 ratio used in most of these freezing tests.

Although the strawberry varieties Auchincruive Climax and Huxley were judged to be superior when frozen to heat-processed, the reverse was true for the variety Royal Sovereign, which shows that individual varieties need careful testing before any general conclusions can be drawn. Up to the present it appears that freezing has no advantages over heat-processing methods either for fruits which are normally cooked before eating, such as blackcurrants and gooseberries, or for pears, but is superior for soft dessert fruits. Frozen blackberries, if cooked before tasting, were preferred to the uncooked fruit.

Summary.

The results of tasting tests on 24 varieties of soft fruits and pears preserved by bottling, canning and deep freezing are given, also 12 varieties of plums preserved by bottling and canning.

The frozen fruit was generally superior to the heat-processed fruit in colour, but frequently inferior in texture or flavour. The best method of preserving varies considerably from one kind of fruit to another.

Acknowledgments.

Our thanks are due to Mr. G. E. Clothier, Miss B. Hart and Miss M. Hooper who were regular members of the tasting panel, and to Miss Hart and Miss Hooper for assistance in the processing.

REFERENCES.

- (1) *Ann. Rept. Campden Res. Stn.*, 1949, 15.
- (2) CRANG, A. and KENDALL, L. *Ann. Rept. Long Ashton Res. Stn.*, 1949, 166.

SUNSHINE AT LONG ASHTON, 1921-1950.

By G. E. CLOTHIER.

A summary of the sunshine records at Long Ashton Research Station from 1921 to 1950 is given in this Report. Records are taken with a Campbell-Stokes sunshine recorder elevated on a roof top 31 feet above ground and well situated to give an unobstructed exposure to the sun at all times of the year except during part of June and July when it is shaded by a hill to the north-west shortly before sunset.

Annual Sunshine.

The average annual sunshine at Long Ashton during the thirty years under review is 1532.2 hours, or 4.20 hours per day. The variations in annual totals show that from 1921 to 1933 every fourth year was very sunny (Fig. 1). This was followed by a succession of six years until 1939 with the totals below normal, the lowest being 1307.3 hours (3.57 hours per day), in 1936, which was very little more than in the most sunless year of all, 1931, when 1305.9 hours were recorded.

Not until 1948 and 1949 was there a recurrence of very sunny years, the total for 1949, 1837.8 hours (5.03 h.p.d.) being not far short of the total of 1880.3 hours (5.15 h.p.d.) for 1921, the sunniest year on record. Two very sunny years in succession, as in 1948 and 1949, have occurred only once previously, in 1921 and 1922 (1920 was a dull year).

A comparison of the two sunniest years, 1921 and 1949, shows that in 1921 the total was made up by high amounts for all months from April to October, except August, while in 1949 there was a more even distribution of excess hours over the months, all but April and September being above normal.

A very sunny March, May, July and September accounted for most of the excess hours in 1929, the third best year, and only January and August were below normal. The inclusion of 1925 as a very sunny year was largely accounted for by the remarkable total of 334.8 hours of sunshine in June.

In the average year, 71% of the total sunshine is recorded from April to September, but in the very dull year of 1931 the total over these months was only 53% of the normal. In 1936, almost equally dull, February, August, October and December were the only months with totals above normal. In 1947, the 63% of an average year's total which is provided from February to July was reduced to 51%, and only a very sunny August prevented the year from being numbered among the very dull ones. In a sunny year, 1948, the summer months, June, July and August, were all deficient in sunshine, but the excess was largely accumulated in March, April and May.

Monthly Sunshine.

Table I shows the total sunshine for each month and year from 1921 to 1950, together with the average hours per day and month.

The monthly averages are also illustrated in Fig. 2, and it will be seen from the diagram that a curve following the average monthly totals rises smoothly from March to June but shows some irregularity on the declining summer months of July and August.

TABLE I.
SUNSHINE AT LONG ASHTON. 1921-1950. MONTHLY AND ANNUAL TOTALS (HOURS).

Year	Jan.	Feb.	Mar.	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Total	Hours per day
1921	24.1	66.5	132.4	226.0	246.4	275.7	278.9	185.4	187.1	172.1	49.5	36.2	1880.3	5.15
1922	46.8	80.9	104.3	191.0	280.9	235.2	199.0	143.8	125.2	129.9	58.0	32.0	1621.0	4.44
1923	50.1	64.8	96.2	132.1	188.2	162.1	185.6	240.2	150.4	82.8	97.0	47.4	1498.9	4.11
1924	64.2	67.5	168.2	167.1	192.3	178.2	217.5	157.5	128.6	86.1	47.4	57.8	1530.4	4.18
1925	44.7	79.6	100.6	176.7	195.6	334.8	211.8	169.2	166.4	103.7	89.6	66.9	1745.6	4.78
1926	40.0	50.3	99.8	130.8	172.7	229.3	177.3	195.7	113.7	93.2	44.3	42.5	1389.6	3.81
1927	46.0	54.8	125.3	182.4	202.0	174.9	175.1	168.0	116.7	103.8	53.0	17.1	1419.1	3.89
1928	66.7	77.7	80.3	118.2	162.6	757.7	292.0	188.1	198.2	112.1	63.9	64.2	1575.7	4.31
1929	36.3	77.7	197.3	170.5	238.7	238.7	238.7	168.8	208.0	102.3	73.0	58.7	1788.8	4.90
1930	53.5	56.1	114.7	134.2	131.1	252.2	197.4	199.3	140.4	102.1	76.9	34.1	1492.0	4.09
1931	62.3	76.2	133.2	<i>704.3</i>	161.2	181.8	<i>724.4</i>	<i>737.7</i>	101.4	114.0	72.3	37.1	<i>7305.9</i>	3.58
1932	47.4	62.3	136.2	121.5	<i>727.6</i>	247.7	148.4	180.3	111.4	102.2	48.6	41.4	1375.0	3.76
1933	78.4	85.2	180.1	178.8	158.7	217.9	245.7	228.8	167.4	92.8	75.2	41.8	1750.8	4.80
1934	57.7	96.5	107.5	124.0	221.0	201.4	264.3	187.1	128.9	<i>62.8</i>	<i>75.8</i>	32.4	1499.4	4.11
1935	42.6	60.8	108.9	126.8	169.3	189.0	251.1	203.2	139.3	82.4	53.4	43.4	1470.2	4.03
1936	35.1	74.8	79.9	130.2	177.3	172.3	132.3	196.8	99.9	103.5	42.0	63.2	1307.3	3.57
1937	40.0	51.7	122.0	114.9	219.9	213.5	136.3	219.1	168.4	78.1	53.5	39.7	1457.1	3.99
1938	39.0	61.7	145.5	205.8	145.3	208.2	137.3	143.2	123.1	106.7	48.7	50.1	1415.6	3.88
1939	36.7	77.5	171.5	180.5	210.5	232.0	144.9	159.5	158.4	112.3	33.1	39.6	1465.8	4.01
1940	66.3	30.8	137.4	113.8	206.0	282.8	187.0	220.1	186.2	82.1	82.3	39.8	1634.6	4.47
1941	35.0	69.3	83.9	114.7	148.8	194.5	222.7	189.6	124.1	115.9	43.8	38.7	1381.0	3.78
1942	40.9	55.9	74.8	200.9	195.5	227.6	192.3	157.4	139.8	85.6	51.2	35.2	1457.1	3.99
1943	57.3	69.1	147.8	169.9	238.3	200.7	200.5	188.0	159.3	119.9	64.1	38.8	1653.7	4.53
1944	30.3	63.7	162.7	138.8	233.7	173.4	130.9	213.7	151.4	105.7	37.2	54.3	1515.8	4.14
1945	63.2	60.4	145.1	204.6	180.0	187.6	162.5	158.4	<i>88.8</i>	99.7	33.7	42.4	1426.4	3.91
1946	45.9	64.3	107.8	212.1	191.2	162.9	211.0	152.0	112.1	63.9	43.7	79.0	1445.9	3.86
1947	53.5	<i>20.8</i>	74.9	154.2	188.6	192.5	179.7	270.8	155.0	108.3	73.2	29.9	1471.4	4.03
1948	38.9	84.9	170.3	211.4	286.3	186.9	183.0	155.4	160.9	98.5	73.1	61.9	1681.3	4.59
1949	57.3	111.5	166.4	231.3	232.7	282.7	273.8	216.9	124.3	114.6	64.4	54.1	1837.8	5.03
1950	37.3	50.6	116.4	159.5	190.9	242.3	183.0	172.5	107.0	93.4	51.5	63.4	1473.8	4.04
Average	47.9	67.1	122.6	159.4	195.3	212.7	196.1	185.7	141.4	100.8	57.1	46.1	1532.3	4.20
Hrs. per day	1.54	2.38	4.09	5.31	6.30	7.09	6.32	5.99	4.71	3.25	1.90	1.49		

Figures in bold type and italics indicate the largest and smallest amounts respectively.

The monthly averages shown as percentages of the maximum duration of sunshine theoretically possible in the latitude of Long Ashton (Table II), show that May, June and August have been proportionally rather more sunny than July, owing to a higher frequency of cloud and/or haze in the latter month.

TABLE II.

AVERAGE MONTHLY AND ANNUAL SUNSHINE SHOWN AS % OF THE THEORETICAL MAXIMUM POSSIBLE IN THE LATITUDE OF LONG ASHTON.

Month	% of possible	Month	% of possible
January	19	July	39
February	24	August	41
March	33	September ..	38
April	38	October	31
May	40	November ..	22
June	43	December ..	19
		Annual	34

Many of the monthly extremes appear in the bright and dull years, as would be expected, and most of the highest monthly values are to be found in the early and later years of the period, with seven of the "sunniest on record" for each month occurring between 1921 and 1929 and four between 1946 and 1948. There is a preponderance of lower values in the mid-period, with six of the "dullest on record" months between 1931 and 1934. The absence of extremes between 1935 and 1945 is noticeable, only two "records," both "lows," falling within that time.

The sunniest month of any was June, 1925, with 334.8 hours (11.2 hours per day). This exceeds the normal monthly total by 122.1 hours, and was 42.8 hours more than the next sunniest month of any, July, 1928, with 292.0 hours (9.4 h.p.d.).

In October, 1921, a total of 172.1 hours (5.55 h.p.d.) was recorded. This total is noteworthy, not only for the amount, which exceeds that of the next sunniest October (in the following year) by 48.2 hours, but also because the deviation from the normal in this month have been otherwise relatively small.

The most sunless month of all was November, 1934, the total of 15.8 hours being 41.3 hours below normal, and 17.9 less than in 1945, the next dullest month.

The variations from the normal of the extreme monthly totals are greater in the positive than the negative direction in all but the winter months. From April to September the totals for the sunniest in each month vary from +46% to +57% of the normal, compared with -29% to -37% for the corresponding dull months.

Daily Sunshine.

Table III shows the distribution of daily sunshine during the twenty years 1931 to 1950, tabulated between stated limits in a manner devised by the Meteorological Office (1).

TABLE III
PERCENTAGE DISTRIBUTION OF DAILY SUNSHINE WITHIN STATED LIMITS BETWEEN 1931 AND 1950.

% Frequency of	Jan.	Feb.	Mar.	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Year
No Sun	41	31	15	9	7	6	5	5	7	13	34	39	18
Little Sun—0.1–3.0 hours	34	34	32	30	24	20	27	24	33	43	40	39	32
Moderate Sun—3.1–6.0 hours	20	22	21	18	21	17	19	21	25	23	18	19	20
Sunny Days—6.1–9.0 hours	4	13	21	19	18	21	21	21	21	18	7	2	16
Very Sunny Days—Over 9.0 hours ..			11	23	30	36	28	29	13	2	0	0	14

The big difference in the frequency of sunless days from October to November and from February to March occurs at a time when the power of the sun is decreasing or increasing rapidly, and during the winter months a thin veil of cirrus or slight haze which would have no effect at other times of the year is sufficient to reduce the intensity of the sun to below the limits of registration by a sunshine recorder.

The greatest number of sunless days in any month was 20 in December, 1927, followed closely by 19 in November, 1934. The highest for January was 17 in 1941, which included nine consecutive days, the longest sequence of sunless days for any month, though there was a similar period from January 26th to February 3rd, 1940. In February, 1947, 18 sunless days were registered.

During the summer months, May to August, the extreme range of very sunny days (over 9.0 hours per day) was from 23 in June, 1925, to 2 in July, 1931. In June, 1921, a total of 21 days included a spell of 13 consecutive days, which, with the last three days of May, provided an unequalled run of 16 very sunny days. The highest total for May, July and August was 18 days in 1922, 1947 and 1949 respectively, while at the other end of the scale, four days in May, 1932, and June, 1948, with five in August, 1922, are the lowest numbers recorded.

Summary.

1. Records of the monthly and annual sunshine at Long Ashton during the years 1921 to 1950 are given.

2. The annual sunshine has varied between 1880.3 hours (5.15 hours per day), and 1305.9 (3.58 hours per day), the average being 1532.2 hours (4.20 hours per day).

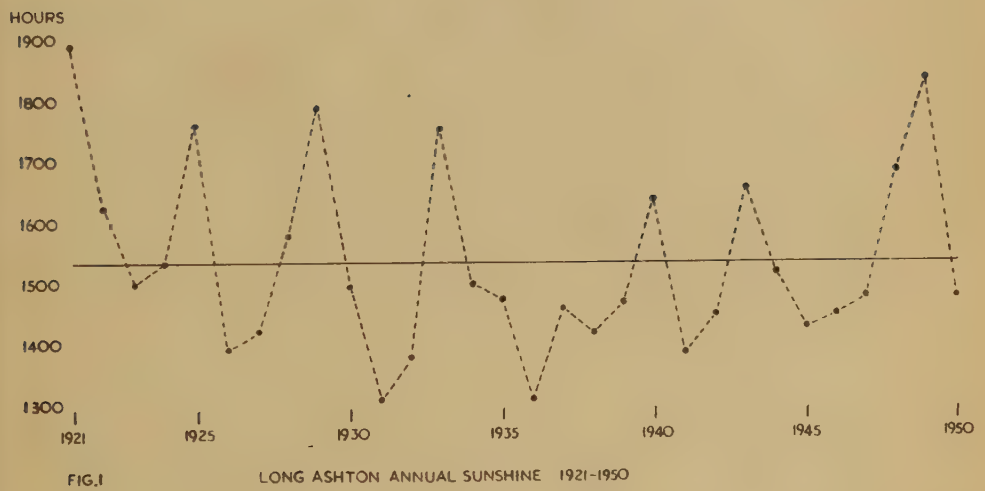
3. The very sunny years were recorded during the first part and the close of the period, while only two years with totals above normal were registered between 1934 and 1947.

4. The sunshine amounts for the extremely sunny months have varied more widely from the normals than the extremely dull ones.

5. During the 20 years ended 1950, the frequency of sunless days was highest in January, and of very sunny days highest in June.

REFERENCE.

- (1) METEOROLOGICAL OFFICE, AIR MINISTRY. Averages of Bright Sunshine (MO 408).



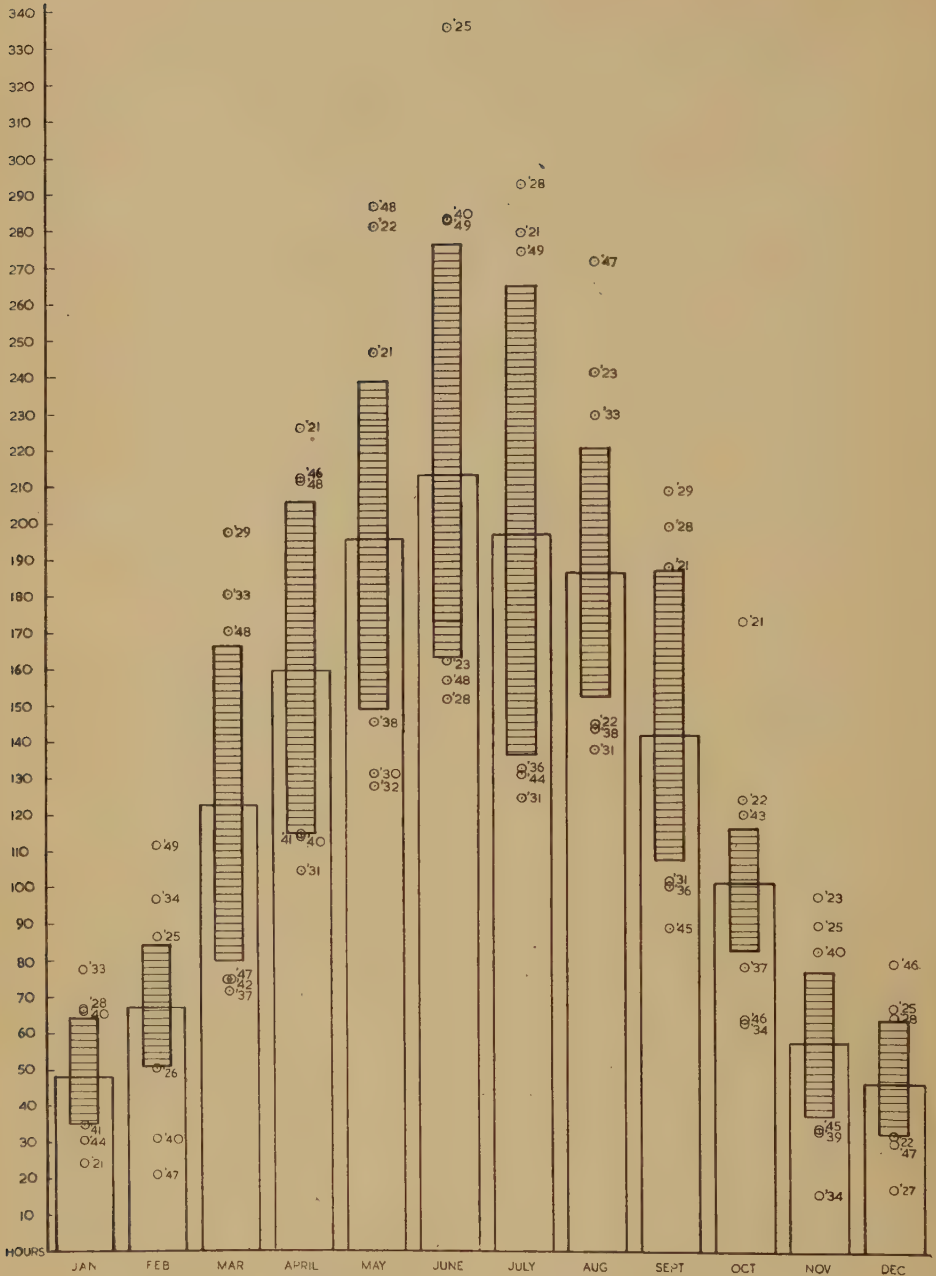


Fig. 2. Monthly Sunshine at Long Ashton 1921-1950.

Outlined columns indicate average monthly sunshine. Superposed shaded columns indicate the range of 80% of the records. Totals above and below the 80% range and the years in which they were recorded are also indicated.

PUBLICATIONS, 1950

BOULD, C.

Methods of applying nutrients to fruit trees. *R.H.S. Fruit Year Book*, 1950, 96-97.

BOULD, C.

The influence of cultural practices on the nutrient uptake of apples. *Trans. 4th Internat. Congr. Soil Sci. Amsterdam*, Vol. 1. 262-265.

BOULD, C., NICHOLAS, D. J. D., POTTER, J. M. S., TOLHURST, J. A. H. and WALLACE, T.
Copper deficiency of fruit trees in Britain. *Nature*, **165**, 920-923.

CRANG, ALICE and STURDY, MARGARET.

Retention of ascorbic acid in preserved fruits. II. *J. Sci. Food and Agric.*, **1**, 252-254.

CROWDY, S. H.

Observations on apple canker. III. The anatomy of the stem canker. *Ann. Appl. Biol.*, **36**, 483-495.

CROWDY, S. H. and WAIN, R. L.

Aryloxyaliphatic acids as systemic fungicides. *Nature*, **165**, 937.

GLEEN, H.

Biological oxidation of iron in soil. *Nature*, **166**, 871.

HEWITT, E. J., AGARWALA, S. C. and JONES, E. W.

Effect of molybdenum status on the ascorbic acid content of plants in sand culture. *Nature*, **166**, 1119-1120.

HOBBIS, E. W.

Fruit growing on the farm. *Progressive Farming*, Vol. II. 1949.

KEARNS, H. G. H. and MORGAN, N. G.

Ground spraying and dusting equipment for crop protection. *British Agricultural Bulletin*, **2**, 415-422.

LUCKWILL, L. C.

How plant hormones aid crop production. *World Crops*, **2**, 17-20. 1950.

LUCKWILL, L. C.

Some virus diseases of fruit trees in England. *R.H.S. Fruit Year Book*, 1950, 84-88.

MARSH, R. W.

Apple disease and growers' problems in England. *N.Y. State Hortic. Soc. Proc.*, 1950, 266-268.

MARSH, R. W.

Report on a visit to Canada and U.S.A. to study research and development in agricultural fungicides, March, 1949 to February, 1950. *Agric. Res. Coun. Rept.*, 12241.

MARTIN, H.

Advances in chemical methods of crop protection. *J. Sci. Food and Agric.*, **1**, 163-167.

MARTIN, H.

The limitations and advantages of the new insecticides. *Agriculture*, **57**, 19-22, 1950.

MARTIN, H.

Chapter on Insecticides : The Pharmaceutical Industry in Germany during the period 1939-45. *B.I.O.S. Survey Report*, **24**, 1950.

MARTIN, H.

The new phosphorus insecticides. *R.H.S. Fruit Year Book*, 1950, 89-91.

NICHOLAS, D. J. D. and FIELDING, A. H.

Use of *Aspergillus niger* as a test organism for determining molybdenum available in soils to crop plants. *Nature*, **166**, 342.

NICHOLAS, D. J. D. and FIELDING, A. H.

Use of *Aspergillus niger* for determining magnesium, copper, zinc and molybdenum deficiency in soils. *J. Science of Food and Agriculture*, **2**, 339-344.

PLANT, W.

A survey of mineral deficiencies in crops on arable land in two English counties. *Empire J. Exp. Agric.*, **18**, 41-48.

PLANT, W.

Soil fertility. *Fertiliser and Feeding stuffs J.*, **36**, 601-4.

PLANT, W.

Nutrition of horticultural crops. *N.A.A.S. Quarterly Review*, No. 6, 69-70; No. 7, 119-120; No. 8, 165-166; No. 9, 22-24.

PLANT, W.

The use of lime and sodium molybdate for the control of whiptail in broccoli. *Nature*, **165**, 533.

PLANT, W.

The relation of molybdenum deficiency to the acid soil complex. *Fourth int. Congr. Soil Sci., Amsterdam*, Vol. II, 148-151.

PLANT, W.

The control of whiptail in broccoli and cauliflower. *Agriculture*, **57**, 130-134.

POLLARD, A.

Vitamins in fruit juices and related products, Chap. X in Recent Advances in Fruit Juice Production. *Bur. of Hort. and Plantation Crops, Tech. Commun.*, No. 21, 125-144, 1950.

POLLARD, A.

Report on 2nd International Congress of Fruit Juice Producers. *Food Manufacture*, Oct., 1950, 405-408.

POLLARD, A.

Wine from the garden. *R.H.S. Fruit Year Book*, 1950, 110-116.

SKERRETT, E. J. and WOODCOCK, D.

Insecticidal activity and chemical constitution. Part II. Synthesis of some analogues of D.D.T. *J. Chem. Soc.*, 1950, 2718-2724.

SKERRETT, E. J., STRINGER, A. and WOODCOCK, D.

Insecticidal action of D.D.T. *Nature*, **165**, 853.

SPINKS, G. T.

Long Ashton Research Station. (Account of Field Day, July, 1950). *Nature*, **166**, 220-222.

WALLACE, T.

The diagnosis of the mineral status of plants, with special reference to deficiencies, excesses and interactions of nutrient elements. *Rept. B.C.S.O. Specialist Conference in Agriculture*. Melbourne, Australia.

WALLACE, T.

Diagnostic des carences minerales chez les vegetaux en particulier par les methodes visuelles. *Actualités scientifiques et industrielles*, **1073**. Hermann et Cie., Paris, 1949.

WALLACE, T.

Diagnosis of soil fertility by visual symptoms of crops. *Trans. 4th Internat. Congr. Soil Science, Amsterdam*, Vol. I, 1950 (Congress Lecture).

WALLACE, T.

The mineral nutrition of crops with special reference to fruit crops. Cawthron Lecture, Nelson, New Zealand, 1949. Cawthron Institute, Nelson, New Zealand.

WILLISON, R. S.

Virus diseases of stone-fruit trees. *Ann. Appl. Biol.*, **37**, 127-130.

